March 1957 · VOL. 16 · No. 1 · PART I

PUBLISHED IN TWO PARTS

Federation Proceedings

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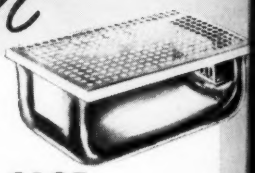

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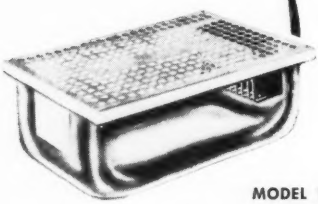

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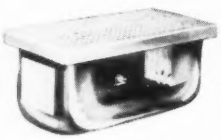

MODEL 103 STANDARD

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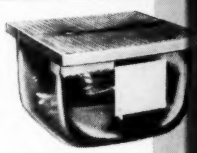

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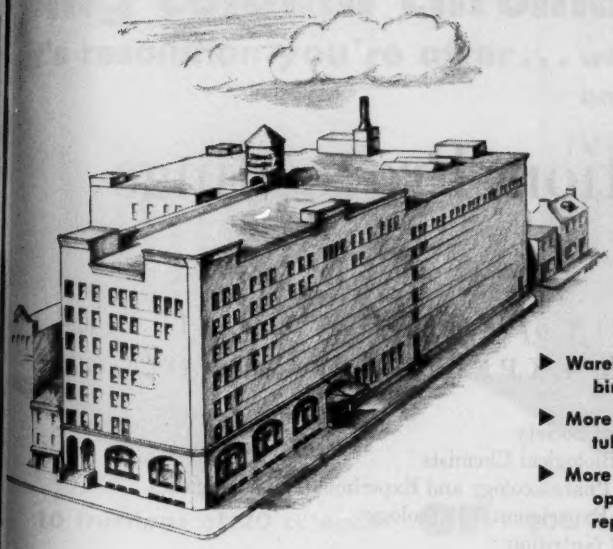

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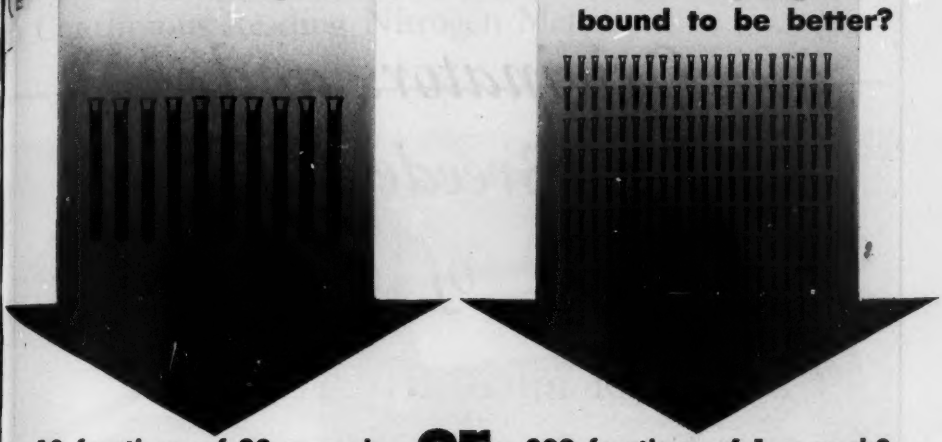

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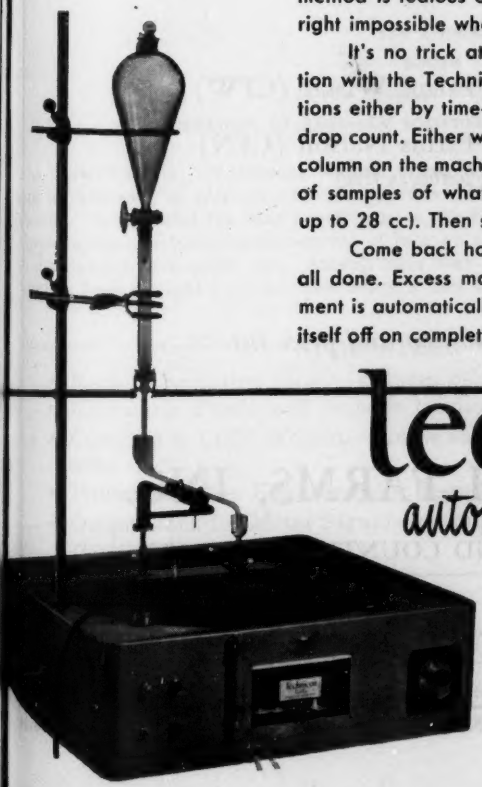

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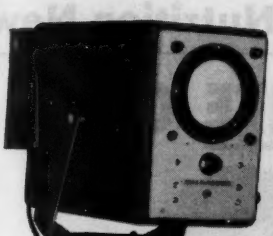

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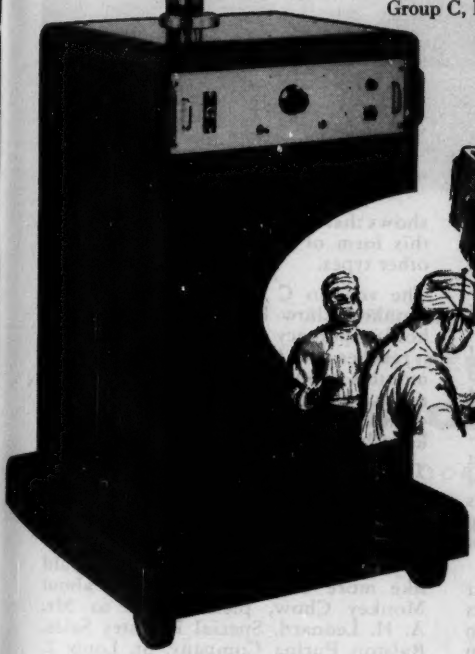

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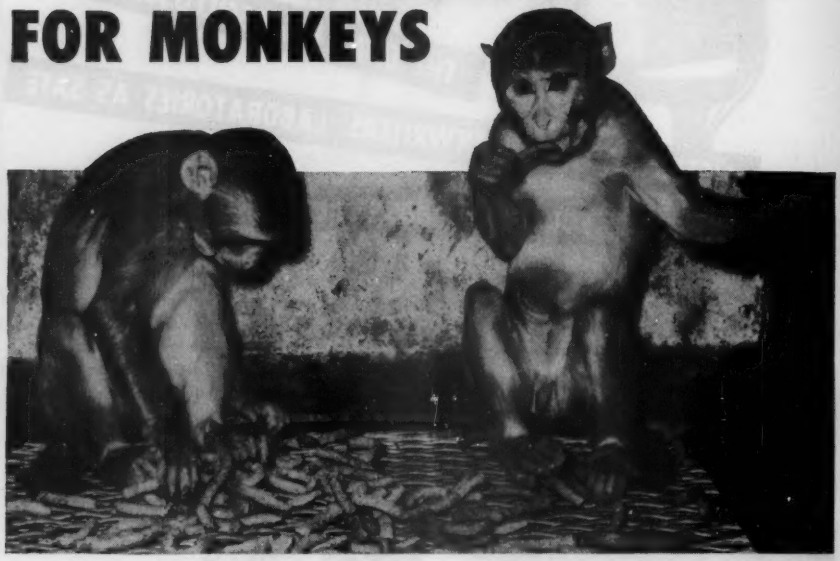

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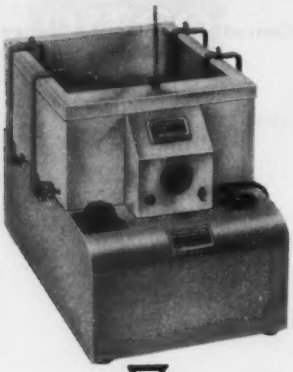

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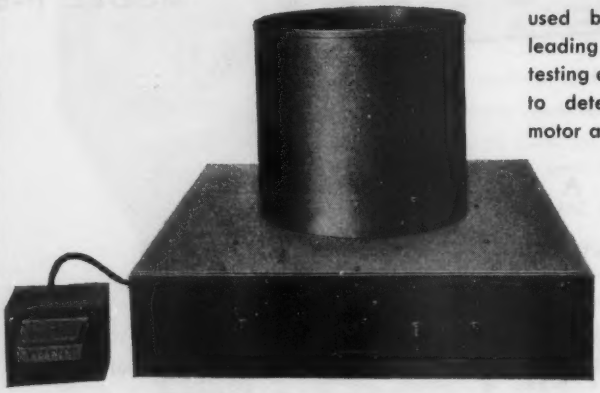

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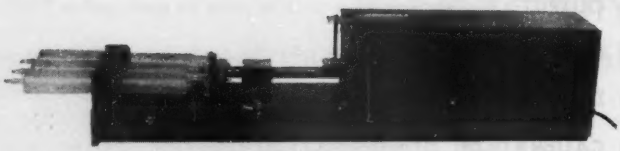

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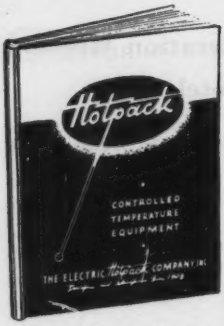

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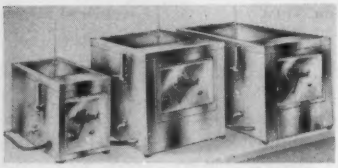

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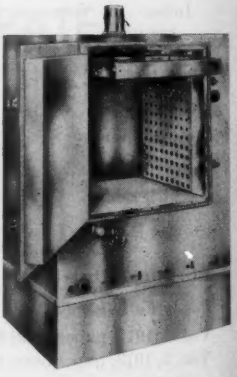

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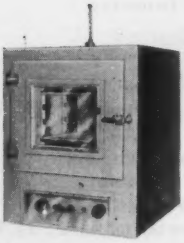

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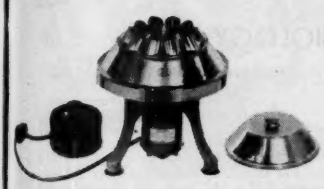

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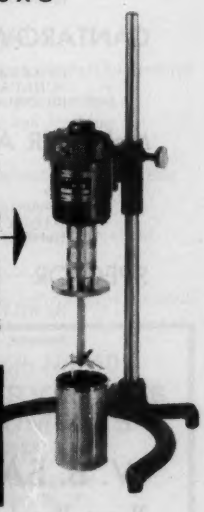

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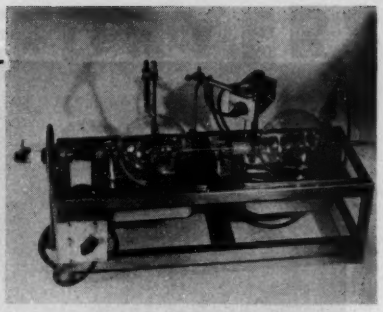

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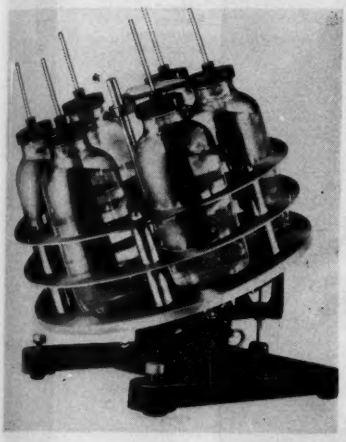

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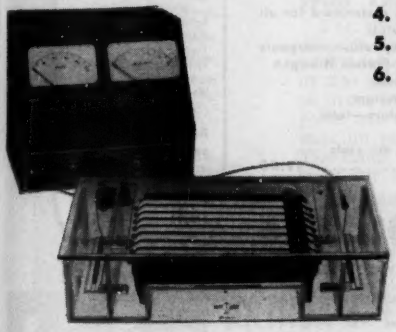

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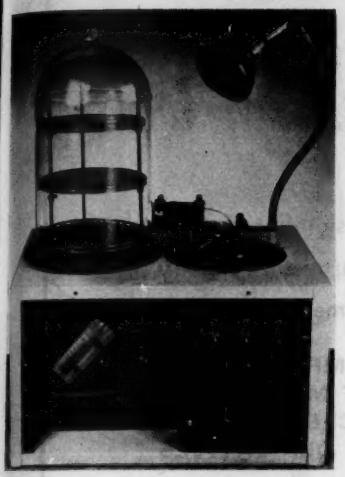

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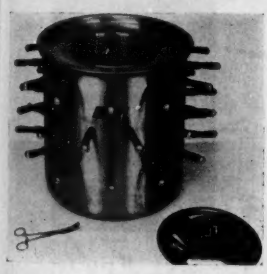

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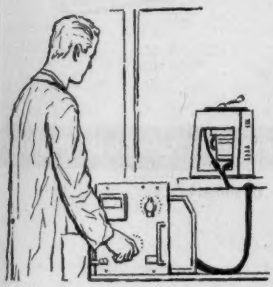

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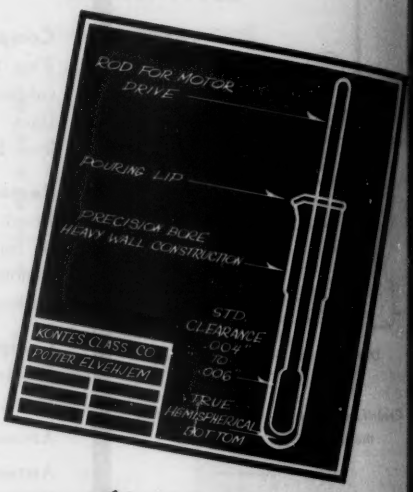

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1. EFFECT OF INDUCED SEIZURE DISCHARGES UPON EVOKED CORTICAL POTENTIALS. A. F. Abdullah* and H. W. Magoun. Univ. of California, Los Angeles, and VA Hosp., Long Beach.

The purpose of this investigation was to determine the influence which seizure discharge exerted upon responses evoked in the anterior sigmoid gyrus of cats. The responses employed were: 1) local cortical, 2) transcallosal, 3) non-specific thalamo-cortical (from intralaminar thalamic nuclei), 4) capsular (from internal capsule) and 5) specific thalamo-cortical (from VPL). Recording was monopolar (with reference to an indifferent lead) from the anterior sigmoid gyrus except with the specific thalamo-cortical response when the active electrode was shifted to the posterior sigmoid gyrus. Superimposed single sweep records were photographed with one oscilloscope and the continuous activity of the cortex monitored on a separate cathode ray tube. Records were made of these potentials before, during and after seizures induced by stimulation of the contralateral anterior sigmoid gyrus. In some instances the effect of Metrazol upon the evoked potentials and upon their response to seizure induction was also assessed. It was found that the local cortical, transcallosal and recruiting responses disappeared during seizure discharge while potentials evoked from stimulation of internal capsule or thalamic relay nucleus persisted. Metrazol whether employed as a convulsant or for activation prior to induction of seizures by cortical stimulation resulted in similar findings although seizure activity was prolonged.

2. KINETICS OF THE PHOTOCHEMICAL CONVERSION OF RHODOPSIN IN SOLUTION. Ralph G. Adams, Henry Linschitz and Donald Kennedy (intr. by Gordon K. Moe). Syracuse Univ., Syracuse, N. Y.

Following exposure to a 150-joule flash of 5 μ sec duration rhodopsin in solution exhibits characteristic changes in transmission. Evidence for increased transmission (bleaching) was obtained at 500, 520 and 540 mμ, but the observed changes were greatest at 520 mμ. There is an initial rapid increase during the first 20 μ sec following the onset of the flash, followed by a continued but slower increase in transmission. Evidence for decreased transmission (absorption) was obtained at 420, 440, 460 and 480 mμ, but the observed changes were greatest at 460 and 480 mμ. There is an initial rapid increase in absorption followed by a continued but slower increase. Measurements were made from photographs of a cathode ray tube metering the light transmitted through the test solution. The onset of the sweep of the cathode ray trace was coordinated with the discharge of the exciting flash. The rhodopsin preparation was placed in a 50 mμ absorption tube and thoroughly evacuated prior to each test. One minute after flash excitation the solution is orange in color and exhibits an absorption maximum at 475 mμ as revealed by a recording spectrophotometer. With time in the dark there is an increase in transmission at 475 mμ and a decrease in transmission at 368 mμ. These changes stop after about 2 hr and the absorption curves obtained show an isosbestic point. Further increase in transmission is produced by exposure to light but both the absorption maxima and the isosbestic point shift toward the ultraviolet.

3. RACIAL VARIATIONS TO A STANDARDIZED COLD STRESS. Thomas Adams, W. R. Beavers, B. G. Covino, R. W. Elsner and R. J. Hock (intr. by W. A. Hiestand). Arctic Aeromedical Lab., Ladd AFB, Alaska.

Previous reports have presented differences in digital vascular tone and extremity cooling rates in response to local and whole body cooling among Negro, Caucasian and Eskimo subjects. In this investigation, 7 healthy, adult male representatives of each racial group were exposed nude for 2 hr in a cold room maintained at 17°C. Rectal, average skin and extremity temperatures, body metabolism, sweating rates and arm and digital plethysmograms were obtained at 5-min intervals throughout the duplicate exposure periods for each individual. After 55 min of exposure both the Caucasian and Eskimo subjects demonstrated a rise in metabolism of approximately 22 Cal/hr/m^2 from control levels of 40 and 55 Cal/hr/m^2 respectively. The Negro subjects showed an increase in body heat production of only 10 Cal/hr/m^2 after 85 min in the cold room, from a control level of 38 Cal/hr/m^2. Although the absolute levels of heat production differed for the Eskimo and Caucasian groups, these subjects responded at the same time with equal increases in metabolic rate. The Negro subjects showed only a slight increase relatively late in the experimental exposures, suggesting a difference in shivering thresholds between these subjects and the other 2 groups. In addition, the Eskimo subjects did not lose as much body heat before shivering and increasing body heat production. These data aid in explaining the reported differences in

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the incidence of cold injury among these 3 racial or ethnic populations.

4. VERATRINE STIMULATION OF CILIARY ACTIVITY. Edward Aiello (intr. by Teru Hayashi). Columbia Univ., New York City.

Cilia on the lateral epithelium of the gills of the marine mussel Mytilus edulis generally exhibit coordinated activity for about 1 hr after excision into sea water. They then become quiescent. Lowered temperature and added orthophosphate (calcium free medium) prolong activity. Increased oxygen tension gives temporary stimulation but shorter duration; i.e. quiescence ensues sooner. Washing has no effect. Veratrine sulfate 0.001% restores normal activity, raises the oxygen uptake, increases dehydrogenase activity and lowers the respiratory quotient. A large number of diverse treatments give only temporary stimulation. Potassium sensitizes the tissue to veratrine. Washing and pH experiments suggest that veratrine acts inside the cell and that washing has no specific effect of its own. Good anaerobic activity has not been observed. Flouride and iodoacetate are inhibitory at 10^{-3} M as are also cyanide, azide and 2,4-dinitrophenol. At 10^{-8} M dinitrophenol augments the stimulation by low levels of veratrine. Initial activity apparently leads to self-inhibition, especially when operating at a fast rate. Veratrine seems to stimulate and regulate the production of utilizable energy in such a way that a high rate of ciliary activity can be maintained. (Supported in part by a PHS Predoctoral Fellowship.)

5. DISAPPEARANCE OF A SOLUBLE NEUROHYPOPHYSIAL PROTEIN AFTER WATER RESTRICTION. R. W. Albers and M. W. Brightman (intr. by W. F. Windle). Lab. of Neuroanatomical Sciences, Natl. Insts. of Health, Bethesda, Md.

Lyophilized neurohypophyses from rats given water ad lib. were extracted successively with ethanol plus hexane (I), and with 50% ethanol-1% acetic acid (III). Solvent system I extracted 19.7% of the total dry weight and III extracted 43.9% ± 1.9% of the residue. Neurohypophyses from hydrated rats and from rats on restricted water intake were submitted to the same extraction procedure. In glands from hydrated rats, 47.3% ± 0.9% of the nonlipid fraction was soluble in III. In material from dehydrated rats, only 22.6% ± 1.0% was soluble in III. To characterize further the labile material, extraction by I was followed by extraction with 70% ethanol-1% acetic acid (II), and then by extraction with III. Fraction I-II remained nearly constant in glands from both hydrated and dehydrated animals, whereas fraction II-III was nearly absent from glands of dehydrated rats. Fraction II-III comprises about 17% of the total dry weight of the neurohypophysis of the hydrated rat. Its nearly complete disappearance after water deprivation is elicited by the same stimulus that invokes secretion of the antidiuretic hormone. This result indicates relatively little contamination of fraction II-III with inert protein. It may thus be analogous to the van Dyke protein isolated from bovine glands or to the neurophysine of Acher.

6. IMMEDIATE EFFECTS OF STRETCH ON MUSCLE CONTRACTILITY. Robert S. Alexander. Dept. of Physiology, Albany Med. College, Albany, N. Y.

Using both striated muscle (isolated frog gastrocnemius) and smooth muscle (dog bladder in vivo), tension development in response to periodic electrical stimulation has been measured after sudden stretch of the tissue. In the unstimulated state, sudden stretch produces an abrupt rise in elastic tension followed by a period of partial tension decay (stress relaxation). Peak responses to stimulation during this period, however, exhibit much less of a decay in absolute tension and, in the case of small stretches of striated muscle, there is actually no decay in the peak contractile tension. In both striated and smooth muscle, therefore, there is a progressive increase in the tension developed in response to stimulation during the period of stress relaxation. This is interpreted as evidence that in the passively shortened unstimulated state, some of the contractile links tend to lock in the shortened state and therefore cannot contribute to the contractile process until an external force, maintained for a finite period of time, serves to pull them free. Such a process can also explain the increase in elastic stiffness which develops in muscles permitted to rest in a shortened state. This concept is essentially a restatement of older ideas which postulate a "catch" mechanism for explaining some aspects of muscle tone. (Aided by a grant from the Life Insurance Med. Research Fndn.)

7. RADIOAUTOGRAPHIC STUDIES OF IRON KINETICS IN THE DOG. Edward L. Alpen, Doris Cranmore* and A. K. Davis. U. S. Naval Radiological Defense Lab., San Francisco, Calif.

One of the most widely applied techniques for estimation of erythropoietic function is the radioactive iron utilization curve. This curve is difficult to interpret in quantitative terms because of effects of pool size and storage effects. Therefore a direct measurement of kinetics of iron movement by radioautography of serial bone marrow specimens has been undertaken. Direct marrow radioautography shows that all erythroid cells, up to the reticulocyte, rapidly become labeled, but at different rates. The youngest cell (erythroblast) is the most active in this regard. Soon after injection of Fe^{59} the erythroblast is the most radioactive cell, but as division and maturation proceeds, the peak of maximum labeling proceeds through the steps of maturation. A series of curves can be drawn demonstrating progression of labeling from erythroblast to reticulocyte and mature erythrocyte. Maturation time for reticulocytes is estimated at a minimum of 30 hr while time for the last identifiable radioactive cell to reach maturity is approximately 7 days. Immature radioactive erythrocytes appear in peripheral blood as early as 4 hr after administration of the label. The number of labeled erythrocytes continues to increase at 0.2-0.4%/day, reaching a peak of 1.5-3.0%. It is suggested that this appearance curve for labeled erythrocytes represents a more direct measure of cellular activity of bone marrow than the curve of peripheral blood total radioactivity.

8. EFFECTS OF GROWTH HORMONE ON CARBOHYDRATE METABOLISM OF HYPOPHYSECTOMIZED DOGS. N. Altszuler,* R. Steele,* J. S. Wall* and R. C. de Bodo. Dept. of Pharmacology, New York Univ. College of Medicine, New York City, and Biology Dept., Brookhaven Natl. Lab., Upton, N. Y.

Previous studies with C^{14} glucose showed that unanesthetized hypophysectomized dogs, in the post-absorptive period, had a smaller than normal body glucose pool and a diminished rate of glucose turnover, i.e. glucose inflow from the liver into the plasma and glucose outflow from the plasma into the tissues. Growth hormone treatment (1 mg/kg/day, i.m., for 4-5 days) in hypophysectomized dogs increased the glucose inflow from the liver into the

plasma and the glucose outflow from the plasma into the tissues. It also increased the size of the glucose pool. Studies on insulin hypersensitivity revealed that an i.v. injection of insulin into hypophysectomized dogs resulted in a greater uptake of plasma glucose by the tissues than in normal animals, and the release of glucose by the liver in response to the hypoglycemia was inadequate, thereby resulting in a more severe and prolonged hypoglycemia. In contrast, in normal dogs, the hypoglycemia evoked a rapid release of glucose by the liver. In growth hormone-treated hypophysectomized dogs the increase in plasma glucose uptake by the tissues following insulin injection was less than before treatment and the response of the liver to the induced hypoglycemia was restored to normal. The observed changes in glucose turnover may explain the ability of growth hormone to raise the plasma glucose concentration, abolish the insulin hypersensitivity and produce diabetes.

9. EFFECT OF HYPOXIA AND ISCHEMIA ON CORTICAL, THALAMIC AND MIDBRAIN RECULAR NEURONS. Vahe E. Amassian. USAF, School of Aviation Medicine, Randolph AFB, Tex.

Cats were anesthetised with Nembutal, Dial, chloralose or prepared under local anesthesia combined with a paralysant drug. Spontaneous activity and activity evoked by forepaw, thalamic or cortical stimulation was observed in the appropriate multiunit records from medial lemniscus, midbrain reticular formation, medial and ventroposterior thalamic nuclei, internal capsule, somatosensory areas I and II and pyramidal tract. Activity of individual cortical and reticular neurons was recorded with micropipettes of 0.5-8μ tip diameter. The effect of nitrogen breathing, low oxygen mixtures, arterial occlusion in the neck or acute pulmonary embolism was tested. Arterial oxygen saturation was determined with a continuously recording oximeter mounted on an arterio-venous shunt and by the Van Slyke method. Spontaneous (including burst) activity in the cortex and medial thalamus practically disappeared before or when the arterial oxygen saturation fell below 10%. Early evoked somatosensory cortical responses, pyramidal responses and evoked activity in single cortical neurons persisted many seconds later. Spontaneous activity was similarly more susceptible to cerebral ischemia than was evoked activity. Activities of individual reticular neurons markedly differed in susceptibility to hypoxia, the activity disappearing either when cortical spontaneous activity disappeared or later. Such behavioral differences may be related to the degree of proximity to the afferent inflow. The latency of evoked discharge usually increased prior to loss of response. Thus, transmission of short delay evoked discharges within the cortex is less sensitive to hypoxia and ischemia than is the subcortical mechanism which drives cortical "spontaneous" activity.

10. EFFECT OF PROTAMINE ON THE NEUROMUSCULAR JUNCTION. E. G. Anderson,* E. A. Schenk* and D. F. Magee. Dept. of Pharmacology, Univ. of Washington School of Medicine, Seattle.

The action of protamine, a weak histamine liberator, on the neuromuscular junction was investigated. Previous work in this laboratory has demonstrated the ability of the histamine liberating compound 48/80 to potentiate the effect of d-tubocurarine on the neuromuscular junction. The cat sciatic nerve-tibialis muscle preparation was used. It was found that 150 mg/kg of protamine produced a partial neuromuscular block. Intra-arterial injections were more effective than intravenous injections. The protamine-induced block appears to be of the non-depolarizing type, since it can be antagonized by edrophonium chloride. A dose of 40 mg/kg of protamine potentiated a d-tubocurarine block and prolonged its action to 3 or 4 times that of a control. Doses of protamine that produced no effect alone caused a strong block when administered following recovery from a d-tubocurarine block. Protamine also antagonized a depolarizing type block induced by decamethonium. The degree of protamine effect on the neuromuscular junction varied in direct proportion to the speed of injection.

11. SOME CIRCULATORY CHANGES IN INJURED GOATS. Rubert S. Anderson, Fred W. Stemler,* Raymond F. McHugh* and Earl B. Rogers.* Biophysics Div., Directorate of Med. Research, Army Chem. Ctr, Md.

Fourteen goats under barbital sodium were severely wounded in a standard manner with explosive pellets. Seven animals were wounded on both hind legs and 7 on 1 hind leg. Erythrocyte and plasma volumes, cardiac outputs and a few blood components were measured before and at 0.5, 3 and 22 hr after the injury. Substantial losses in both erythrocyte and plasma volumes occurred which were accounted for only in part by the measured external blood losses in spite of the fact that some compensation for lost plasma would be expected. Judging by the decrease in plasma protein concentrations, compensation apparently occurred to about the same extent as following simple hemorrhage. The average ratio of body hematocrit to venous hematocrit was normally above the value of 1.0 reported for the dog while after adrenaline the ratio approached the value reported for splenectomized dogs. Cardiac outputs decreased sharply at one-half hour post-blast in both the singly and doubly wounded animals, but later the decrease in the singly blasted animals tended to lag behind the other group in time. At one-half hour the average blood pressures in both groups had dropped to about one half, reflecting, largely, the average decrease in cardiac output.

12. HYPERPOTASSEMIA, CARDIAC ARRHYTHMIAS AND VENTRICULAR FIBRILLATION RESULTING FROM RAPID REVERSAL OF HYPERCARBIA. Philip W. Andrews,* Frank E. Adrian* and Archer S. Gordon. Dept. of Clin. Science, Univ. of Illinois Med. School, Chicago.

Cardiac arrhythmias and ventricular fibrillation have been reported following rapid reversal of hypercarbia in animals. This process has been implicated also in human cases of ventricular fibrillation occurring during or immediately after surgery. In this study, nembutalized dogs rebreathed into a bag for periods of 90 min. The dogs were then rapidly hyperventilated with room air. EKG's and carotid artery blood pressure were recorded continuously; serial arterial plasma pH, CO_2 content and potassium were determined as often as every minute during periods of rapid change. The plasma potassium reached peaks of 50% above the control levels within 10 min after the onset of rebreathing. It then fell gradually to values slightly above the control and remained there until the CO_2 was blown off, at which time it reached a second peak more rapidly and higher than the first. The height of the second peak correlated with the speed of CO_2 elimination. The EKG was essentially unchanged until the CO_2 was removed, when ominous arrhythmias developed in 50% of the dogs and ventricular fibrillation in 20%. The severe

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arrhythmias occurred in dogs having the most rapid removal of CO_2. The pCO_2 reached levels of over 300 mm Hg, but could be brought to subnormal levels within 2 - 3 min by hyperventilation. This gradual accumulation of endogenous CO_2 and its subsequent rapid removal results in changes similar to those described after breathing 30-40% CO_2 for 2-4 hr, or with sudden reversal of hypercarbia during and immediately following surgery.

13. QUINIDINE AND QUINIDINE-LIKE COMPOUNDS ON HYPOTHERMIC VENTRICULAR FIBRILLATION. E. T. Angelakos* and A. H. Hegnauer. Boston Univ. School of Medicine, Boston, Mass.

Quinidine and quinidine-like compounds were tested for ability to alter incidence of spontaneous hypothermic ventricular fibrillation (VF). Among controls, 75% fibrillated at a mean temperature of 20.4°C. In addition to quinidine (2-10 mg/kg), other drugs tested and their per kg dosages were: procaine amide (10-50); Ambonestyl, MC 4112, (40-50); pyrilamine (5); thephorin (5); diphenhydramine (5-10) and its diethyl analog S-45 (5); quinacrine (5); Ro 2-7302/4 (2-10), and Ro 2-5803 (2). At 10 mg/kg quinidine was effective in preventing spontaneous hypothermic VF in all but one of 20 trials. The effect diminished progressively with lower dosage, but was still significant at 2 mg/kg. Ambonestyl and Ro 2-7302 had significant but limited effect even with larger doses. Procaineamide and all other compounds tested were ineffective. It would appear that the quinidine protection against spontaneous hypothermic VF is fairly specific, since the property does not appear in most compounds which are adjudged highly "effective" when tested by conventional screening methods for antiarrhythmic or antifibrillary activity. Quinidine (10 mg/kg) was also tested for its capacity to prevent VF which occurs in 100% of untreated hypothermic dogs subjected to right ventriculotomy (22° ± 2). Under these conditions quinidine offered only limited protection, less than that observed with Ambonestyl in similar tests. The results suggest that quinidine acts mainly as an antiarrhythmic, while Ambonestyl appears to be mainly antifibrillary.

14. ABSORPTION OF OLEIC ACID FROM ILEAL LOOPS. John Annegers. Dept. of Physiology, Northwestern Univ. Med. School, Chicago.

Oleic acid alone or with adjuncts was introduced every 15 min in controlled quantities into the upper end of 24-inch Thiry-Vella terminal ileal loops in 4 unanesthetized dogs. The effluent was collected from the lower fistula, having drained freely throughout the perfusion period. After flushing with saline, the lipid content of the effluent was measured and the amount of lipid which disappeared during 3- and 6-hr tests was calculated. When oleic acid alone was given, lipid absorption was about 2 gm/hr. As saline was added to the perfusion mixture, the rate of lipid absorption decreased and was nearly directly proportional to the initial concentration of oleic acid in the mixture. In subsequent tests, this relationship of absorption rate to initial lipid concentration was used as a basis for comparison. Desiccated ox bile in amounts ranging from 2-36 mg bile salt/cc of perfusion mixture failed to increase the rate of lipid absorption. However, the rate of absorption was increased by including sodium bicarbonate in the perfusion, by Nembutal anesthesia and by atropine administration. These procedures tended to decrease the volume of the effluent. Absorption rates up to 8 gm of oleic acid per hour were obtained in atropinized dogs.

15. EFFECT OF DEXTRAN ON BLEEDING TIME, VASCULAR FRAGILITY AND PLATELET THROMBUS FORMATION IN THE HAMSTER. Kenneth A. Arendt,* Herbert J. Berman* and George P. Fulton. Dept. of Biology, Boston Univ., Boston, Mass.

Intravenous administration of dextran (Laros, average MW 75,000) in 55 hamsters and Dextraven (Benger, average MW 230,000) in 24 hamsters resulted in prolongation of bleeding times, increase in vascular fragility and delay in platelet thrombus formation. Twenty-four hours after single i.v. injections of 6 ml of Laros dextran, the average bleeding time was 324 (± 71, S.D.) sec; average petechial count, 137 (± 17); and the average platelet count, 7.45×10^5 ($\pm 1.05 \times 10^5$)/cu mm. The averages in a comparable number of untreated hamsters were, respectively, 106 (± 17) sec, 81 (± 16) petechiae, and 7.71×10^5 ($\pm 1.44 \times 10^5$) platelets/cu mm. Platelet thrombi formed in cheek pouch vessels in 40% of 15 Laros-infused hamsters following topical application of thrombin solution (25 NIH units/ml, Parke, Davis) for 15 min as opposed to 65% of 17 controls. The average bleeding time, petechial and platelet counts, and results with topical thrombin at 24 hr after single 6-ml i.v. injections of Dextraven were 326 (± 74) sec, 107 (± 22) petechiae, 6.35×10^5 ($\pm 1.5 \times 10^5$) platelets/cu mm, and 0%. Both dextrans increased the number of white cells sticking to the venous endothelium in the cheek pouch. The bleeding time was determined by a tail-puncture method. The degree of vascular fragility was indicated by the number of petechiae produced in 1 hr by a standard amount of moccasin venom injected into the cheek pouch. (Supported by the Dept. of the Army, OSG, and grant H-902 (C6), Natl. Heart Inst., PHS.)

16. EFFECTS OF SYMPATHOMIMETIC AMINES ON PULMONARY CIRCULATION. Domingo M. Aviado, Jr., Horacio R. Estrada* and Aldo N. Corbascio.* Lab. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia.

The intravenous injection of various sympathomimetic amines caused a rise or fall in pulmonary arterial pressure depending on the accompanying changes in pulmonary blood flow (PBF) and in pulmonary vascular resistance. PBF was measured as outflow from one lobar vein (and returned to the dog by a pump) whereas local effect on lung vessels was derived from a lobe perfused at a constant flow (with mixed venous blood derived from a donor dog). All pressor amines (in doses sufficient to induce systemic hypertension and cardiac slowing) caused an initial reduction in pulmonary arterial pressure and PBF. Atropinization or cervical vagotomy eliminated such a response. Instead, the local effects of the drug on the lung vessels and the heart became apparent. These can be grouped into 5 patterns, namely: a) increased PBF and pulmonary vasoconstriction (levarterenol, epinephrine, metaraminol, hydroxyamphetamine); b) increased PBF and pulmonary vasodilatation (isoproterenol, mephentermine, methamphetamine); c) increased PBF with no local effect on lung vessels (ephedrine, cyclopentamine); d) decreased PBF and pulmonary vasoconstriction (phenylephrine); e) decreased PBF with no local effect on lung vessels (methoxamine). Although some amines tested did not affect the lung vessels locally all of them either increased or decreased PBF.

17. AN EXTRACTABLE FACTOR IN PHOTOSYNTHETIC PHOSPHORYLATION. M. Avron and A. T. Jagendorf (intr. by W. D. McElroy). McCollum-

Pratt Inst. and Biology Dept., Johns Hopkins Univ., Baltimore, Md.

Spinach chloroplasts have been used to study the light dependent process of photosynthetic phosphorylation reported by Arnon et al. (Biochem. et Biophys. Acta 20:449, 1956). The activity of these chloroplasts is markedly reduced by repeated washings, and can be restored by adding back concentrated wash water. Evidence will be presented concerning the properties and function of the extracted material.

18. UTILIZATION OF KETONE BODIES IN ASCORBIC ACID DEFICIENCY. Habeeb Bacchus, Albert F. Debons* and William Kishi.* Dept. of Physiology, George Washington Univ. School of Medicine, Washington, D. C.

Previous studies from this laboratory have shown that liver slices of ascorbic acid-deficient guinea pits produce significantly smaller amounts of ketone bodies than do slices from control animals. In the presence of a fatty acid substrate (octanoate) however, the ability of liver slices of ascorbic acid-deficient animals to produce ketone bodies exceeds that of slices from control (pair-fed) animals. The total liver lipid content of both groups of animals was similar. It seems probable that in ascorbic acid deficiency there is a lesion in the formation of fatty acids from endogenous fat. In spite of the proven decreased spontaneous ketogenesis in ascorbic acid deficiency, the resting levels of ketone bodies in these animals are similar to those in control animals. Ketone body utilization studies were done in ascorbic acid-deficient and pair-fed control animals. The data suggest that, in addition to the decreased spontaneous ketogenesis in ascorbic acid deficiency, there is probably a lesion in ketone body utilization in this condition. These data might be related to alterations in cholesterologenesis and steroidogenesis in ascorbic acid deficiency. (Aided by Grant A-852 from Natl. Insts. of Health, PHS, and by a contract with the Office of Naval Research.)

19. MESENTERIC LYMPHATIC ADJUSTMENTS DURING SHOCK. S. Baez, A. Carleton* and I. Forbes.* Dept. of Medicine, Cornell Univ. Med. College and the New York Hosp., New York City.

Changes in the basic dynamic behavior of the mesenteric lymphatics and in the rate of lymph flow have been observed to occur regularly during experimental hemorrhagic shock in rats. The lymphatics undergo pronounced compensatory and decompensatory adjustments recalling those seen in metarterioles and precapillaries of the same region. During the period when the animal is recoverable by transfusion, the lymphatic vessels exhibit progressive enhancement of spontaneous motion and of sensitivity to topically applied epinephrine, with increasing leucocytic population; the over-all diameter of the vessels decreases and lymph flow is well maintained. Reversal of these features occurs upon prolongation of drastic hypotension. Coincident with spontaneous uptake of blood from the reservoir, the lymph vessels show increasing numbers of erythrocytes and zones of localized atonic distention. Upon transfusion of the shed blood, exaggerated spontaneous motion of the lymphatics is observed; the flow increases but now consists of blood instead of lymph. Lymph vessel adjustments after sublethal drum trauma are of a compensatory type, compatible with survival, whereas following lethal drumming, the lymphatics invariably appear atonically distended and resemble those seen in irreversible hemorrhagic shock. These observations have been extended to rats pretreated with Dibenzyline and G-D 131, which protect against shock, and to rats made resistant to trauma by progressive sublethal drummings. These findings demonstrate that the lymphatics do not constitute a static system but instead participate in over-all readjustments of the splanchnic area during stress.

20. ANTIHISTAMINICS IN PREVENTION OF ACUTE ADRENAL FAILURE. Carleton H. Baker* and John W. Remington. Dept. of Physiology, Med. College of Georgia, Augusta.

It has become axiomatic that 1) the adrenalectomized animal is considerably more sensitive to various forms of stress than the intact animal, and 2) that the stress acts in a non-specific manner to precipitate a circulatory collapse because of some derangement of the cardiovascular apparatus. A striking illustration is the prompt pressure fall which follows an infusion of about 100 cc normal dog plasma into an adrenalectomized dog on maintenance therapy. A rapidly developing hemoconcentration, edema, intestinal congestion and lumenal bleeding suggested a capillary defect. We find that pretreatment with 5 mg/kg Benadryl prevents these changes and protects life. A single stage bilateral adrenalectomy is usually fatal within 48 hr, the collapse often being associated with intestinal bleeding. Recovery rates are significantly better after the administration of Benadryl. One precipitating factor is nerve trauma, since survival also follows procaine infiltration of the splanchnic nerves or the spinal cord. In contrast, Benadryl did not lower the sensitivity of 72-hr adrenalectomized rats to morphine, sodium pentobarbital or cold. However the fatal dose of the first two was not appreciably less than with intact animals. The action of Benadryl against the circulatory collapse following intestinal manipulation, which also involves afferent nerve stimulation, is under study. (Supported by grants from PHS and Life Insurance Med. Research Fund.)

21. ALDOSTERONE EXCRETION FROM HYPOPHYSECTOMIZED DOGS WITH THORACIC INFERIOR VENA CAVA CONSTRICTION. Wilmot C. Ball, Jr.,* Robert C. Bahn, M. Jay Goodkind* and James O. Davis, Natl. Heart Inst., Bethesda, Md., and Mayo Clinic, Rochester, Minn.

In dogs with thoracic inferior vena cava (TIVC) constriction and ascites, the rate of urinary aldosterone excretion is greater than normal and Na retention is almost complete (Am. J. Physiol. 187:45, 1956). During the first 5-10 days following hypophysectomy in dogs with TIVC constriction either 1) urinary aldosterone excretion decreased to normal and Na excretion increased, or 2) urinary aldosterone output decreased but remained above normal while Na excretion remained low. In the latter situation, the rate of aldosterone excretion returned to normal during the next few weeks and a natriuresis occurred. The decrease in aldosterone excretion was not prevented by administration of ACTH during the post-hypophysectomy period. In 1 hypophysectomized dog with TIVC constriction given ACTH, IVC pressure was increased by further constriction of the TIVC; aldosterone excretion returned to the high pre-hypophysectomy level and Na retention again became almost complete. In another group of dogs, hypophysectomy was performed first and the TIVC was constricted 6-8 wk later. Two of these dogs then developed increased urinary aldosterone excretion, marked Na retention and ascites. The adrenal cortex in these 2 dogs was markedly atrophic, but the atrophy was limited to the 2 inner layers; the zona glomerulosa appeared normal.

22. CORONARY FLOW STUDIES WITH DIAL-URETHANE ANESTHESIA COMPARED WITH CHLORALOSE ANESTHESIA. T. A. Balourdas* and J. C. Scott. Inst. for Cardiovascular Research and Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.

Coronary flow studies were performed using the N_2O desaturation method with 80% oxygen and 20% N_2O in normal mongrel dogs. Average results of 20 or more experiments with each anesthesia indicate similar values in each group for: coronary flow/100 gm of left ventricle/min, left ventricular work, cardiac index, mean arterial blood pressure, arterial oxygen vol. %, LV. oxygen consumption. Heart rate was markedly decreased with chloralose, and coronary vasular resistance and left ventricular efficiency were increased. The coefficient of correlation between coronary flow and mean arterial blood pressure was low with Dial-Urethane anesthesia but relatively high with chloralose. Flow-heart rate correlation was much higher with Dial-Urethane. Atropine increased coronary flow, heart rate and LV. oxygen consumption markedly in each group while left ventricular efficiency was decreased in both categories but somewhat less with chloralose. It may be assumed that the decreased heart rate with chloralose is due to increased vagal tone from the carotid sinus reflex. The failure to obtain a decrease in flow under these circumstances with approximately the same mean arterial blood pressure constitutes negative evidence for vagal vasoconstriction of coronary flow with atropine in both groups may be explained independently of the vagal blocking action of this drug. In general an increased coronary sinus blood flow is associated with an increased LV. oxygen consumption and an increased heart rate. (Supported in part by the Natl. Heart Inst. Grant, PHS, #H. 2003 R.)

23. EFFECT OF ACETAZOLEAMIDE (DIAMOX) AND AMMONIUM CHLORIDE ON ACID-BASE BALANCE IN PULMONARY EMPHYSEMA. Anthony J. Barak and Herbert P. Jacobi (intr. by Arthur L. Bennett). Med. Research Lab., VA Hosp. and Univ. of Nebraska College of Medicine, Omaha.

Previous investigators have reported that Diamox administration brings symptomatic relief to patients with pulmonary emphysema. The improvement has been attributed to the observed decrease in arterial pCO_2 (Am. J. Med. Sci. 229:193, 1955). The present investigation deals with a comparison of the effects of NH_4Cl and Diamox on pH, pCO_2 and bicarbonate concentration of arterial plasma in pulmonary emphysema. Adult male patients were used in this study. Fasting blood was used throughout and samples were drawn and analyzed prior to and after the administration periods. Two groups of patients were used: group I received each 10 mg of Diamox/kg/day for 7 days. Group II received each 6 gm of NH_4Cl/day for 7 days. NH_4Cl produced a drop in pH and bicarbonate concentration equal to that produced by Diamox. The average lowering of pCO_2 in the case of NH_4Cl was equal to the average lowering in pCO_2 in those patients on Diamox. These data suggest that Diamox and NH_4Cl have the same end result on pCO_2 in arterial blood. We can, therefore, assume that the alveolar pCO_2 will be lowered in emphysematous patients with either treatment. In view of these findings the symptomatic relief found with Diamox and not with NH_4Cl cannot be attributed to lowering of alveolar pCO_2. The improvement found with Diamox must be due to some other effect or mechanism not yet understood.

24. INTRARENAL ARTERIAL INFUSION OF ALDOSTERONE AND ANTIDIURETIC HORMONE IN THE NORMAL AND ADRENALECTOMIZED DOG: ELECTROLYTE AND WATER EXCRETION. A. C. Barger, R. D. Berlin* and J. F. Tulenko.* Dept. of Physiology, Harvard Med. School, Boston, Mass.

The development of a technique for chronic catheterization of one renal artery, with collection of urine from each kidney individually, has afforded the opportunity of studying the effects on water and electrolyte excretion produced by aldosterone and antidiuretic hormone infused directly into the renal artery of normal and adrenalectomized dogs. The present investigation clearly indicates the local renal action of antidiuretic hormone on water excretion and the rapidity of its action. No effect on sodium excretion was observed with ADH in these normal dogs on a water diuresis. In the normal dog intrarenal aldosterone produced a unilateral kaluresis but no demonstrable anti-natriuresis, or effect on water secretion. In the same catheterized dogs, following recovery from adrenalectomy, small doses of aldosterone infused into the renal artery produced a unilateral anti-natriuresis. The unilateral kaluresis was of the same magnitude as that observed in the normal state. In the normal and adrenalectomized animals the effect of intrarenal aldosterone was apparent only after a delay of 30-60 min, and the maximum effect was noted after 2-3 hr. These temporal relations were the same with both intrarenal and intravenous infusions.

25. RENAL RESPONSE TO MAGNESIUM LOADING IN THE DOG. Earl S. Barker,* John K. Clark and J. Russell Elkinton. Dept. of Medicine, Univ. of Pennsylvania, Philadelphia.

Magnesium sulfate or lactate was infused intravenously into 7 dogs for periods up to 5 hr at average rates in μ Eq/kg/min of 30-50 (highest 120). Urinary magnesium excretion rate (UV_{Mg}) increased rapidly to but not above approximately 50-65% of the filtered load of magnesium ($Filt._{Mg}$) calculated as the product of plasma Mg and glomerular filtration rate (GFR) measured by exogenous creatinine clearance. Using the "filtrable" fraction of plasma Mg, the ratio $UV_{Mg}/Filt._{Mg}$ reached approximately 1.0 and in some instances exceeded 1.0. This would indicate tubular secretion of Mg, but the excess was seldom more than 10%. As plasma Mg concentration was increased the ratio of Mg clearance to GFR did not show a clear-cut "self-depression" which would have suggested "unmasking" of saturation of a tubular excretory process. If estimations of "filtrable" Mg are correct, increases in UV_{Mg} tended to approximate changes in $Filt._{Mg}$ suggesting the possibility that for Mg the kidney may operate at or near saturation of active tubular processes. Deterioration of the animal usually prevented $Filt._{Mg}$ from rising above about 400 μEq/min. Artificial respiration permitted larger doses and higher $Filt._{Mg}$. Nevertheless the ratio $UV_{Mg}/Filt._{Mg}$ did not show further rise. Similarly sodium lactate, mercury or chronic prior loading did not appear to increase the ratio. While the data do not exclude a three-component system including tubular secretion, they may be explained by filtration and reabsorption alone. (USPHS Support H-405, H-340.)

26. THYROXINE MAINTENANCE OF INCUBATED KIDNEY SLICE METABOLISM. S. B. Barker and R. H. Lindsay.* Dept. of Pharmacology, Univ. of Alabama Med. Ctr., Birmingham.

After an initial measurement of oxygen consumption

of thyroidectomized rat kidney slices at 37°C, incubation has been carried out at 5°C without shaking. At intervals of 24, 48 and 72 hr, the flasks were re-equilibrated at 37° for metabolism determinations. With Krebs' Ringer-phosphate-glucose as medium, oxygen consumption fell to 57% of the starting level after 24 hr, 31% after 48 hr, and 21% after 72. Added thyroxine (5-10 g/ml) had no effect at the start, but metabolism was better maintained after incubation, being 79%, 64% and 56% of the start value after 24, 48 and 72 hr, respectively. Tris buffer was no better than phosphate, nor was glycine-NaOH alone. Addition of thyroxine to the Ringer-glycine-glucose system led to 101% maintenance of respiration at 24 hr, 82% at 48, and 71% at 72. Neither glycylglycine nor diglycylglycine was better than phosphate, either alone or with thyroxine. Substitution of alanine for glycine led to 99% maintenance after 24 hr without thyroxine. Addition of the hormone gave a value 22% above the start level, which was still 114% at 48 hr. Out of many amino acids and related materials evaluated in place of alanine in this setup, the following have been outstanding: β-alanine, threonine, N-acetyl-alamine, β- and γ-amino-n-butyric acids, leucine, proline, asparagine, ornithine. Participation of certain specific nitrogenous materials in this thyroxine maintenance of surviving kidney tissue is thus indicated. (Supported by research grants from the American Cancer Soc. and the Smith, Kline and French Fndn.)

27. PHYSIOLOGY AND CLINICAL MANAGEMENT OF ATHEROSCLEROSIS AND CORONARY HEART DISEASE. Broda O. Barnes and Robert W. Barnes.* Denver, Col.

Atherosclerosis can be produced experimentally by cholesterol feeding to rabbits or chickens, by thyroidectomy in sheep or goars, or by thyroidectomy and cholesterol feeding in dogs. In all instances, thyroid administration inhibits the arterial damage and reduces the blood cholesterol. We are studying the blood cholesterol and the development of atherosclerosis in the rat thyroidectomized by radioactive iodine. We have also studied the human blood cholesterol in over 400 unselected adults from an active general practice. The following impressions have been obtained. Less than one quarter of the general population have elevated cholesterols. The occurrence of coronary heart disease or stroke in this group has not been in those patients with the highest blood cholesterols. Elevated blood cholesterols are accompanied by other signs of thyroid deficiency, and thyroid therapy returns the blood cholesterols to the normal range. Even a diet containing an excessive amount of fat has not produced a hypercholesterolemia over a period of 17 years in the presence of adequate thyroid function. The ancestors of many of the patients with elevated cholesterols came from the goiter regions of the world. Although the descendants are susceptible to infections, they are now living long enough to develop atherosclerosis. Relieving the thyroid deficiency seems a more physiological approach to hypercholesterolemia than the highly publicized low-fat diet.

28. PENICILLIN INHIBITION OF MALONATE AND ACETATE METABOLISM OF PSEUDOMONAS FLUORESCENS P-6. Robert C. Barnett and G. L. Morrow (intr. by E. L. Porter). Carter Physiological and Med. Physics Labs., Univ. of Texas Med. Branch, Galveston.

Penicillin G in concentrations of 400-800 units/ml competitively inhibit hydroximate formed from acetate and malonate when autolyzed Pseudomonas fluorescens (P-6) cells is used as the enzime system. Growth in 24 hr at 30°C and hydroximate inhibition is essentially the same over the same range of penicillin concentrations. Penicillin either hydrolyzed in IN HCl at 100°C for 10 min or treated with penicillinase for 3 hr at 30°C show neither growth nor hydroximate inhibition when malonate or acetate is used as substrate. To possibly ascertain the cite of penicillin action, experiments following both hydroximate formation and CO_2 evolution were performed in the presence and absence of inhibitor and at varying concentrations of cells, ATP, Mg^{++} and CoA. A linear logarithmic relation exists between penicillin inhibition of hydroximate formation and milligrams of autolyzed cells added. This was considered to indicate that enzymatic inhibition occurred either by interference with CoA and/or ATP activation of substrate or combination of penicillin directly with ATP, CoA or Mg^{++}. The rate of CO_2 evolution with the complete system - containing CoA, ATP and Mg^{++} - in the presence of penicillin is reduced to that which is attained in the absence both of added penicillin and ATP. The primary effect of penicillin on the complete system is to increase the lag phase and not the rate; whereas in the absence of ATP (the greatest penicillin effect) the absolute rate of CO_2 production is reduced. Penicillin, however, had a negligible inhibitory effect on CO_2 production in the absence of CoA or Mg^{++}. From these results it was concluded tentatively, that penicillin acts at a step previous to CO_2 production inhibiting ATP activation of CoA.

29. GENERATION OF PROTHROMBIN ACTIVITY WITH MITOCHONDRIA OR WITH CATHEPSIN. Marion I. Barnhart (intr. by Ernest D. Gardner). Dept. of Physiology and Pharmacology, Wayne State Univ., Detroit, Mich.

The capacity possessed by mitochondria to generate prothrombin activity is a recent discovery. The present study was concerned with cellular particulates and the specific enzymes which may direct regeneration of prothrombin activity from an "inactive" prothrombin preparation. Conditions have been found for obtaining high yields of prothrombin with the use of mitochondrial systems. Moreover, it was observed that cathepsin possessed the property of generating prothrombin activity. Preparations containing several different cathepsins as well as preparations more potent in cathepsin B and C were utilized. Cathepsin B, which is largely localized on mitochondria, has proved the most efficient proteinase in genesis of prothrombin activity in solutions of pH 7.5-8.1, the range for similar activity with mitochondrial preparations. Mitochondria and cathepsin accelerate destruction of prothrombin activity when acid conditions prevail. Whether or not cathepsin concentrates and mitochondria inactivate prothrombin or generate the activity from "inactive" prothrombin depends on the conditions selected. Since parallel results were obtained with mitochondria and cathepsin in these protein reactions, it is possible that cathepsin of the mitochondrion is the active agent in generation of prothrombin activity from a precursor.

30. AGE DIFFERENCES IN CELLULAR METABOLISM OF VARIOUS TISSUES OF RATS. C. H. Barrows, Jr.,* M. J. Yiengst,* N. W. Shock and B. F. Chow.* Gerontology Branch, Natl. Insts. of Health, Bethesda, Baltimore City Hosps. and Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.

Ten young male and 10 young female rates (12-15) months) were compared with a like number of old animals (24-27 months) for changes in the cellular

metabolism of kidney, heart and liver. All animals were bred in our colony and reared under standard conditions. The results indicate that the agewise decrease (10%) in the rate of endogenous oxygen uptake of kidney slices of female rats approximates the decrement in the amount of protoplasm as estimated by the concentration of DNA, RNA and protein N. In contrast, the agewise change in the concentration of succinoxidase of kidney homogenates (18%) exceeded the loss of protoplasm. These findings were not as striking when the kidneys of male rats were examined. However, analysis of heart tissue also indicated that a decrease (10%) in the concentration of the enzyme in both male and female rates exceeded the loss of cellular mass in the tissue (1-2%). No significant changes were found in any of these measurements in liver. These results suggest that liver, a tissue which retains regenerative potential even in old age, exhibits agewise changes to a lesser extent than do kidney and heart. In the latter organs, age changes have been observed which cannot be explained entirely on the basis of a reduction in cellular mass in the aged tissues.

31. EFFECT OF MODERATE AND SEVERE DECOMPRESSION ON BLOOD GLUCOSE OF ADRENALECTOMIZED DOGS. Jeanne Q. Barry,* Frederick P. Ferguson and Dietrich C. Smith. Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore.

Acute exposure of intact dogs and chronically adrenalectomized dogs maintained on DCA or cortisone or in moderate adrenal insufficiency to a simulated altitude of 30,000 ft (moderate decompression) or to altitudes above 40,000 ft (severe decompression) resulted in an elevated blood glucose concentration. With moderate decompression, glucose of intact dogs increased from an initial level of 109 mg% to 139, 130 and 120 mg% after 30, 60 and 90 min at 30,000 ft. In comparable experiments on DCA-maintained adrenalectomized dogs, the glucose rose from 87-102, 97 and 92 mg%. There was no statistical difference between the response of the 2 groups. In cortisone-maintained dogs, however, the glucose concentration rose from a predecompression value of 113-120 mg% after 30 min and remained at that level throughout the duration of decompression. Although the absolute increase was not large, it was highly significant. In moderately insufficient dogs, the glucose rose dramatically from an average initial value of 144 mg% to decompression values of 214, 192 and 204 mg%. With acute exposure of cortisone-maintained dogs to severe decompression stress, glucose concentration more than doubled, an increase similar to that observed in intact dogs. The data would appear to minimize the importance of the adrenal glands in the mobilization and/or utilization of glucose during decompression stress.

32. ENERGY COST OF BREATHING DETERMINED WITH A SIMPLIFIED TECHNIQUE. R. G. Bartlett, Jr. and Heinz Specht. Natl. Insts. of Health, Bethesda, Md.

The ventilatory volume was self-regulated at various levels by requiring the subject to rebreath through various dead space volumes at a predetermined breathing rate. The dead space was inserted between the subject and a Sanborn Metabulator by which the oxygen consumption was measured. With this very simple apparatus consistent results were easily and quickly obtained. The pulmonary minute volume and O_2 uptake at higher ventilatory volumes are expressed as increments over the resting levels, which appeared to reduce the scatter of the points on a two dimensional plot, and therefore produced a simplification of the curve of best fit. Extrapolation of such a visually fitted curve for a single subject showed the cost of resting ventilation to be .5 cc/l. The energy cost of breathing at 50 l. ventilation was .8 cc/L, and that at 80 l. 2.0 cc/l.

33. LIPID SEQUESTRATION ON CARBOXYLATE EXCHANGE RESIN AND ARTERIAL ELASTIC TISSUE. W. H. Batchelor (intr. by Walter Bauer), R. W. Lovett Mem. Labs., Massachusetts Gen. Hosp., Boston.

Human blood serum loses up to about half of its cholesterol in passing through a column of carboxylate resin (Amberlite IRC-50, 100-200 mesh, Na-H cycle at pH 7). Evidence is presented that the reactive lipid consists of the beta lipoprotein, and that sequestration of this material is accompanied by splitting off of a part of the lipid from lipoprotein. Pressure filtration of serum through excised arteries is known to produce lipid deposits in intima and media (Wilens, Science 114:389, 1951). Elastic lamella and surrounding matrix are under study for lipoprotein reactivity comparable to that shown by carboxylate resin. The spread of serum lipids under pressure from an injected intralamellar depot shows 2 histologic features that have been singled out for chemical definition: 1) a controlling effect of matrix on distribution of lipids, and 2) altered lipid reactivity in the region of ruptured lamellae. (Supporting grant received from The Helen Hay Whitney Fndn., N. Y.)

34. EFFECT OF DIFFERENT PATTERNS OF LOCAL EXERCISE ON PERIPHERAL BLOOD FLOW. A. C. Bauer,* E. R. Vanderhoof* and C. J. Imig. Depts. of Physiology and Orthopedic Surgery, State Univ. of Iowa, Iowa City.

Volume blood flow was measured through the forearms of normal young adult subjects by means of a technique of venous occlusion plethysmography before, sometimes during and following different patterns of local exercise. The exercises employed were isotonic and isometric handgrips of various intensities and durations. The subjects were under basal conditions for all experiments. During an isometric handgrip of low intensity the blood flow was greater than the pre-exercise level of flow. During more intense handgrips the blood flow was less than pre-exercise flow. The amount of decreased blood flow varied directly with the intensity of the exercise. The amount of post-exercise hyperemia also varied directly with the intensity of the exercise. The effect of local exercise on blood flow is determined by the type, intensity and duration of the exercise employed.

35. UPTAKE OF ACETOACETIC ACID BY DIAPHRAGMS FROM CONTROL AND ALLOXAN DIABETIC RATS. Clarissa H. Beatty, Rose Mary Bocek* and Edward S. West.* Biochemistry Dept., Univ. of Oregon Med. School, Portland.

It has been postulated that a decrease in peripheral utilization of ketone bodies is a contributory factor in diabetic ketosis. Many workers have demonstrated that extrahepatic tissues utilize ketone bodies in the absence of insulin. However, no careful comparison of peripheral utilization by diabetic and nondiabetic tissues has been made. Therefore, we compared the disappearance of acetoacetic acid from Krebs phosphate medium, pH 7.4, containing hemidiaphragms from control rats and rats made diabetic with alloxan

3-11 wk previously. The average uptakes of acetoacetic acid at substrate levels of 3 and 6 mM/l. by diaphragms from 20-hr fasted control rats was 94.1±5.4 S.E. μg/100 mg/hr (n = 10) and 119±8.6 μg/100 mg/hr (n = 12) and by diaphragms from 20-hr fasted alloxan diabetic rats was 65.6±8.0 (n = 5) and 64.2±10.3 (n = 5). The uptake of acetoacetic acid (3 mM/l.) by diaphragms from fed nondiabetic rats was 86.7±2.4 μg/100 mg/hr (n = 14) and from fed alloxan diabetics, 67.0±6.8 μg/100 mg/hr (n = 10). This represents a 30-45% decrease in acetoacetic acid uptake by diaphragms from fasted diabetics and a 20% decrease by diaphragms from fed diabetics. Diaphragms from 2 fasted rats diabetic for over 6 months but in good clinical condition showed acetoacetic acid uptakes of 10 and 5 μg/100 mg/hr at substrate levels of 3 and 6 mM/l. (Aided by PHS grant A-213.)

36. REGIONAL VARIATION IN THE RATIO $\dot{V}_A/FRC$ AS A FACTOR IN ARTERIAL HbO_2 UNSATURATION. Aaron A. Bechtel (intr. by Mary A. Maxfield). Physiology Dept., Hahnemann Med. College, Philadelphia, Pa.

It is accepted that regional $P_{A_{CO_2}}$ and regional R vary with regional $\dot{V}_A$ when regional $\dot{Q}c$'s are equal. This depends on the implicit assumption that the same FRC undergoes greater dilution by the greater $\dot{V}_A$ so that end-capillary blood tensions are different. It is self-evident that such dilution differences could be produced by ventilating unequal regional FRC's with the same $\dot{V}_A \cdot P_{A_{CO_2}}$ and R would be different in the regions with unequal FRC even though $\dot{V}_A$ and $\dot{Q}c$ were the same in both. In terms of the equations for gas exchange, the following comments are pertinent. In any steady state $\dot{V}_{CO_2}$ leaving the blood equals $\dot{V}_{CO_2}$ leaving the lungs:

$$\dot{V}_{CO_2} = \dot{Q}c\,(C\bar{v}_{CO_2} - Cc'_{CO_2}) = \frac{(\dot{V}_A\,P_{A_{CO_2}})}{.864}$$

(where Cc'_{CO_2} is end-capillary blood CO_2 concentration). It follows that

$$P_{A_{CO_2}} = .864\left(\frac{\dot{Q}c}{\dot{V}_A}\right)(C\bar{v}_{CO_2} - Cc'_{CO_2}).$$

The graph of the logarithmic curve of $\dot{V}_A/\dot{Q}c$ ratio variation on the O_2-CO_2 diagram implies that $P_{A_{CO_2}}$ (along with $P_{A_{O_2}}$ and R) is uniquely determined by $\dot{V}_A/\dot{Q}c$ (or $\dot{Q}c/\dot{V}_A$) — given uniform $C\bar{v}_{CO_2}$ and permeability. However, it is evident from equation 2 that $P_{A_{CO_2}}$ and Cc'_{CO_2} may vary without change in $\dot{Q}c/\dot{V}_A$ (e.g. with change only in $\dot{V}_A/FRC$). Since for subdivisions of the lungs $P_{A_{CO_2}}$ difference implies disproportionate $P_{A_{O_2}}$ difference (because of dissimilarities in the dissociation curves), the regional R's may likewise be different, without difference in $\dot{V}_A/\dot{Q}c$ ratio. Variation in regional FRC is thus a factor contributing to unsaturation of arterial Hb with O_2. (Supported in part by grant H-1688 of the American Heart Inst.)

37. ESTIMATION OF LEAN BODY WEIGHT FROM SKELETAL (X-RAY) DIAMETERS. A. R. Behnke. U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Matiegka's (1921) principle relating bone weight (BW) to a product of stature (h) and the squared value of the average of 8 transverse skeletal diameters (TD) (elbows, wrists, knees [femur], ankles) may be formulated as, $BW = h\,(\bar{M}\Sigma 8\ TD)^2\ k$, and used also to estimate lean body weight (LBW) from the same dimensions. On 22 Navy men (h, 177.7 cm; W, 78.2 kg; ages 21-52) the mean value for fat-free body W of the group was 61.7 kg (approx. 63.4 kg LBW) determined by Siri, Donner Laboratory, from both total body water (tritium) and helium dilution body volume techniques. Substituting in the equation, 63.4 for BW, and 6.58 for $\bar{M}\Sigma 8$ TD, the computed value of k is 8.24. The coefficient of correlation for LBW calculated from x-ray TD's and LBW (Siri technique) was .81, somewhat lower than that (r = .83) for LBW based on anthropometer measurements of trunk and extremity diameters. Barring appreciable dysplasia between extremities and trunk, it is possible because of the constant size of the healthy skeleton throughout adult life, to utilize the x-ray measurements to estimate body and external bone size (not necessarily bone weight since the cortex of bone decreases in size with age). Nevertheless, if bone W is 12 (our previously calculated value) to 15% of LBW, then the corresponding values for k in Matiegk's formula are 0.987 to 1.24, incredibly close to his predicted (but not experimentally determined) range of 1-1.2.

38. PHYSIOLOGICAL BASIS OF TASTE PSYCHOPHYSICS. Lloyd M. Beidler. Div. of Physiology, Florida State Univ., Tallahassee.

A theory of the mechanism of taste receptor stimulation has been previously developed in detail (J. Gen. Physiol. 38:133, 1954). This theory accounts for much of the quantitative data obtained from electrophysiological investigations of the taste responses of many different species of animals. This same theory has now been applied to certain psychophysical properties of human taste sensations. A simple taste equation relating the magnitude of taste sensation to the concentration of the chemical stimulus can account for the relationship between the just noticeable difference threshold (jnd) and the stimulus concentration when either sodium chloride or sucrose is chosen as the stimulus. The so-called "absolute threshold" of a substance is dependent upon 3 variables. Two of these are characteristics of the stimulus and the third is dependent upon the psychophysiological state of the animal.

39. SOME PSYCHOPHYSIOLOGICAL EFFECTS OF A TRANQUILLIZING AGENT. F. B. Benjamin, K. Ikai* and H. E. Clare.* Dept. of Physiology, Univ. of Pennsylvania Med. School, Philadelphia.

In twenty-four subjects the effect of 10 mg of preclorperazine (Compazine, S.K.F.) was compared with that of 10 mg of phenobarbital and with a control group which did not receive any drugs. Taking each subject as his own control the following differences were found between the group receiving the tranquillizing agent and the other 2 groups: the group receiving preclorperazine had a higher skin resistance and a lower galvanic skin response to pain. Tests primarily involving mental performance gave a barely significant increase of the number of mistakes. Reaction time was prolonged, while time estimation and sensory perception were not changed. The amplitude of the patellar reflex was decreased and tests primarily involving muscular coordination showed a lower performance. In exercise the increase of heart rate was slightly more pronounced while oxygen consumption showed a marked increase over the 2 control groups.

40. CHEMICAL AND ELECTROCARDIOGRAPHIC EFFECTS OF ALKALI METALS. T. E. Bennett,

M. E. Bender and R. Tarail (intr. by F. R. Griffith). Roswell Park Memorial Inst., Buffalo, N. Y.

Individual intravenous infusions of 0.15M RbCl, KCl, CsCl, and 0.30M LiCl were given to pentobarbitalized dogs at 0.50 ml/kg/min. After controls, blood samples were taken at frequent intervals and at death; continuous electrocardiograms (lead II) were recorded. RbCl infusions contained 1 mc of Rb86; equilibration with K42 (constant ratio of counts per mEq. of serum K) was performed by injecting 2 mc of K42 20 hr prior to infusion. Expected recession of P wave and pronounced auricular-ventricular and intraventricular conduction delays were produced by K, Rb, and Li. Rubidium, however, also caused striking ventricular hyperirritability; but conduction delays were slight after cesium, which produced both marked supraventricular and ventricular hyperirritability. Can rubidium effects be explained by resultant elevation of serum K? Despite significant increases in serum potassium (radioassay) during rubidium infusions these increases were substantially smaller than increases produced by comparable potassium infusions. Furthermore the entire series of electrocardiographic disturbances produced by rubidium invariably occurred at concentrations of serum potassium lower than associated with even the onset of major electrocardiographic alterations produced by comparable potassium infusions. Therefore elevations of serum K, alone, do not account for rubidium intoxication although a contributory role has not been excluded. Incidentally, apparent validation of K42 as a measure of K39 during rubidium loading is demonstrated by insignificant changes in serum specific activity following equilibration with K42 and administration of a KCl infusion having the same specific activity as the serum.

41. CALORIMETRIC DETERMINATION OF FREE ENERGY AND ENTROPY CHANGES. T. Benzinger. Naval Med. Research Inst., Bethesda, Md.

By means of direct microcalorimetry, the driving forces of reversible biochemical reactions, ΔF, ΔH, and $T\Delta S$ may be determined without chemical analysis and without the low temperature physics and large quantities of reactants required for third law entropy determinations. Let two different quantities of heat, Q_1 and Q_2 [cal/mole], be observed in 2 separate experiments upon mixing reactants A + B (for Q_1) and C + D (for Q_2) with the enzyme catalyzing the reversible reaction $A + B \rightleftharpoons C + D$. Then the heat of reaction is $\Delta H = Q_1 - Q_2$, and the free energy, $\Delta F^\circ = -RT \ln (Q_1/-Q_2)^2$. For a reaction of any number of participants,

$$\Delta F^\circ = -RT \ln \frac{(Q_1/(Q_1 - Q_2))^{r+s+t} \cdots [R]_o^r [S]_o^s [T]_o^t \cdots}{(-Q_2/(Q_1 - Q_2))^{a+b+c+\cdots} [A]_o^a [B]_o^b [C]_o^c \cdots}.$$

Some advantages of our procedure in comparison with chemical determinations of equilibria are a) the substitution of one method, calorimetry, for any conceivable number of chemical analytical procedures; b) the fact that calorimetric analysis cannot possibly disturb the equilibrium state, since the measurement is completed when equilibrium is attained; c) the fact that ΔH and ΔS follow simultaneously from the quantities measured for ΔF, namely Q_1 and Q_2; d) the fact that calorimetry is not disturbed, as are some chemical methods including radioactive tracers, by the presence of excessive quantities of reactants with minute quantities of products. The measurement of energy cannot possibly observe the presence of any particles. It responds only to the combined action and counteraction of the direct and the reverse process. It has therefore been successfully applied to a seemingly irreversible reaction, glutamine hydrolysis, whereby the free energy of ATP hydrolysis could be determined (Proc. Natl. Acad. Sci., in press).

42. EFFECT OF THIOSULFATE ON THE RENAL TUBULAR TRANSPORT OF SULFATE IN THE DOG AND IN THE AGLOMERULAR GOOSEFISH. Fredrik Berglund and Roy P. Forster (intr. by William H. Forbes). Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass., and Dept. of Zoology, Dartmouth College, Hanover, N. H.

The effect of various inorganic anions on the renal tubular reabsorption of inorganic sulfate was examined in female mongrel dogs. Sodium salts of bicarbonate, ferrocyanide, phosphate, chloride and thiosulfate were given i.v. in varying doses together with sodium sulfate and creatinine. Of these, thiosulfate depressed sulfate-Tm most markedly. The effect was immediate, occurring within 15 min after injection. Thiosulfate: creatinine clearance ratios were close to 1.0 in most experiments, but it is known that thiosulfate is both reabsorbed and secreted by the renal tubules of the dog. In the aglomerular goosefish (Lophius americanus), on the other hand, sulfate is actively secreted by the renal tubules. Thiosulfate was found to depress the excretion of sulfate to about one fourth of control values. Thiosulfate was itself secreted by the tubules and seemed to replace the sulfate in the urine. These experiments indicate that thiosulfate shares the transport mechanism for sulfate in the renal tubules. This holds true both when the sulfate is transported from the tubular lumen to the blood, as in the dog kidney, or in the opposite direction, as in the goosefish kidney. The presence of endogenous sulfate in plasma of both dogs and goosefish makes it difficult to test whether sulfate also can depress the transport of thiosulfate.

43. EPINEPHRINE, NOR-EPINEPHRINE AND CORONARY BLOOD FLOW. Robert M. Berne. Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Open-chest pentobarbitalized dogs and dog fibrillating heart preparations were employed. The left coronary artery and the coronary sinus were cannulated and coronary perfusion pressure was kept constant at any desired level by a pump perfusion system. Mean and phasic coronary blood flow (CBF), aortic, and coronary perfusion pressures and coronary sinus (C.S.) pO_2 were optically recorded. Single injections of epinephrine or nor-epinephrine from 0.125-5.02 µg into the left coronary of the fibrillating heart induced an initial decrease in CBF followed by a large increase. The latter was associated with reduction of C.S. pO_2. An increase in C.S. pO_2 was not observed. Alterations in intramyocardial or left intraventricular pressure could not account for the initial decrease in CBF. During potassium-induced cardiac arrest epinephrine and nor-epinephrine elicited only a constrictor response. Continuous infusion of epinephrine or nor-epinephrine in the fibrillating and the beating heart produced a greater percentage increase in myocardial oxygen consumption than in CBF. In experiments on the beating heart, C.S. pO_2 decreased, early systolic flow decreased, and in several experiments an initial decrease in maximum diastolic flow was observed. These experiments suggest that epinephrine and nor-epinephrine are primarily coronary vasoconstrictors and that the constrictor action may be masked by the vasodilation which occurs in association with the stimulatory effect of these drugs on the myocardium.

44. MECHANISM OF GLUCOSE ENTRY INTO YEAST CELLS. Jay Boyd Best (intr. by C. Ajmone-Marsan). Dept. of Neurophysiology, Walter Reed Army Inst. of Research, Washington, D. C.

The equation relating rate of entry of a substrate to its external concentration has been previously derived for an enzyme encapsulated by a semi-permeable membrane. If A is the cell surface area, h the membrane permeability, c defined as l/Ah and V_m and k are the Michaelis and Menten constants of the enzyme in situ then V_m, k and c will be the parameters characterizing the encapsulated enzyme system. A minimum χ^2 procedure for obtaining maximum likelihood estimates of V_m, k and c and the variances of these estimates from kinetic data on living cells was derived. Tests of significance for changes in these parameters from application of a drug or inhibitor were constructed. Rates of glucose uptake were measured as a function of glucose concentration for control and uranyl inhibited cell suspension of baker's yeast. The minimum χ^2 procedure was used to analyze the data. A highly significant difference in the membrane parameter c, without significant differences in V_m and k, was observed. The value of c found from the control data is too large to be attributed to diffusion gradients in the aqueous medium surrounding the cell. These results indicate 1) the uranium inhibition is accomplished entirely by a depression in membrane permeability; 2) the membrane permeability is essentially constant over a range of 0-300 mg% glucose; 3) the passage of glucose through the membrane occurs by diffusion rather than via an enzymatic carrier.

45. EFFECT OF HYPOGLYCEMIA AND AMPHENONE ON ADRENAL SECRETION OF EPINEPHRINE AND NOREPINEPHRINE. J. E. Bethune, A. Goldfien, M. S. Zileli and R. H. Despointes (intr. by G. W. Thorn). Dept. of Medicine, Peter Bent Brigham Hosp., Boston, Mass.

The method of Weil-Malherbe and Bone as modified by Aronow and Howard has been used for the measurement of epinephrine and norepinephrine. Hypoglycemia (15-30 mg%) was produced in 8 dogs by the intravenous administration of crystalline insulin (1 μ/kg body wt.). The adrenal venous concentration of epinephrine increased 70-fold from 0.024 μg/min to a peak average of 1.68 μg/min and fell abruptly when 50% glucose was infused. Norepinephrine increased only 5-fold from an average of 0.038 μg/min to 0.19 μg/min and similarly fell with the termination of hypoglycemia. Denervation of the adrenal blocked this increased production of catecholamines. A concomitant slight rise and fall of epinephrine and norepinephrine occurred in peripheral venous blood with a much smaller increment of epinephrine to norepinephrine than in adrenal venous blood. In 2 animals in which muscular activity was stimulated, norepinephrine in the periphery increased to higher levels than did epinephrine. Five dogs were given 500 mg of amphenone intravenously 45-60 min after the administration of insulin; a rapid fall in 17-hydroxycorticosteroid concentration of adrenal venous blood resulted but there was no change in the usual response of epinephrine and norepinephrine. Hypophysectomy did not alter the response to hypoglycemia and ACTH given to the hypophysectomized dog did not enhance the production of catecholamines.

46. PLASMA CHOLESTEROL DEPRESSANT FACTOR IN CORN OIL. J. M. R. Beveridge, W. F. Connell and G. A. Mayer (intr. by G. H. Ettinger). Depts. of Biochemistry and Medicine, Queen's Univ., Kingston, Canada.

In previous experiments in which homogenized formula diets of known composition were utilized it was shown that diets high in corn oil led to lower plasma cholesterol levels than did rations devoid of fat (Circulation 12:499, 1955). Kinsell et al. (Am. J. Clin. Nut. 4:285, 1956) and Bronte-Stewart et al. (Lancet 1:521, 1956) have concluded that the plasma cholesterol depressant effect of such oils may be explained on the basis of the high degree of unsaturation. Recently, we obtained evidence suggesting that the plant sterols present in such oils might be at least partly responsible (Circulation 14:484, 1956). In order to gain further information on this possibility, 57 normal male subjects consumed a homogenized ration free from fat for a period of 8 days. They were then divided into 6 groups which were continued for another 8 days on the following diets: FF, fat-free; 1, 60% of calories from corn oil; 2, fat-free plus the amount of total plant sterol in diet 1 provided as beta sitosterol; 3, 30% of calories from butter; 4, 30% of calories from butter fat plus 30% from corn oil; 5, 30% of calories from butter fat plus the amount of total plant sterol in diet 4 provided as beta sitosterol. No other food was eaten during the experimental period; the only other items that were permitted were water, clear tea, and clear coffee. The results demonstrated unequivocally that the plasma cholesterol depressant effect of corn oil depends to a large extent upon its sterol content.

47. ACTION OF INSULIN ON RELEASE OF FATTY ACIDS FROM TISSUE STORES. Edwin L. Bierman* and Vincent P. Dole. Hosp. of the Rockefeller Inst., New York City.

The concentration of non-esterified fatty acids (NEFA) in plasma falls sharply after an injection of insulin (Dole. J. Clin. Invest. 35:150, 1956). Presumably insulin either limits the release of fatty acids from depots or increases the rate of their removal from blood. To test these alternatives, palmitic acid (previously shown to be a major constituent of NEFA), labeled with C^{14}, was absorbed into homologous plasma and injected intravenously into fasting rabbits. After the initial rapid drop of plasma activity during the first 30 min, the specific activity of NEFA acquired a relatively steady value (corresponding to an apparent dilution space of 100-200 plasma volumes). Insulin (0.1 U/kg, i.v.) given at this time caused the expected fall of NEFA to about one half the control value, but failed to reduce the concentration of C^{14} palmitic in the same fraction or to alter the rate of expiration of C^{14}-O_2. As a result, the specific activity of NEFA was increased reciprocally to the fall of total NEFA. If it is assumed that the portion of labeled palmitic acid that extracts and titrates with NEFA serves as a valid tracer for this group of fatty acids, it follows that insulin limits the release of free fatty acids from tissue stores rather than accelerating the removal of NEFA from plasma, after the native and the tracer materials have been mixed. This interpretation is consistent with the demonstration by Lossow, Brown and Chaikoff (J. Biol. Chem. 220:839, 1956) that insulin retards the breakdown of labeled tripalmitin, and with clinical studies showing marked elevations of NEFA in patients with the accelerated fat metabolism of diabetic acidosis.

48. EFFECT OF BODY TILT, PRESSURIZATION OF LOWER BODY AND AUTONOMIC BLOCKADE ON THORACIC AORTA BLOOD FLOW IN MAN DURING THE VALSALVA MANEUVER. Newton C. Birkhead,* Hiram W. Marshall,* H. J. C. Swan and

Earl H. Wood. Mayo Fndn. and Mayo Clinic, Rochester, Minn.

Congestive heart disease and autonomic nervous system disorders are known to modify the characteristic cardiovascular response of the human subject to the Valsalva maneuver. To study this response further, after a full inspiration an expiratory pressure of 40 mm Hg was maintained against a closed airway for 15 sec by healthy male subjects 1) in the 10° head-up position, 2) in the 10° head-down position, 3) in the 10° head-up position with a g-3A antiblackout suit inflated to 150 mm Hg and 4) in the 10° head-up position after partial ganglionic blockade with intravenous tetraethylammonium choloride (5 mg/kg). Intravascular pressures were recorded and blood flow in the thoracic aorta was measured continuously by the constant-rate indicator-infusion method (Am. J. Physiol. 183:621, 1955). Mean control thoracic aortic flow in the 10° head-up position was 4.2 l/min. Minimal value during the Valsalva maneuver averaged 30, 37, 57 and 23% of control values for 3 subjects under conditions 1, 2, 3 and 4, above, respectively. The beneficial effect of body pressurization and 10° head-down tilt is believed due to the increase in "ambient" central circulatory pressures prior to the Valsalva maneuver, while the detrimental effect of tetraethylammonium chloride is associated with interference with compensatory reactions, chiefly on the arterial side of the circulation.

49. ACETYLCHOLINE TURNOVER IN A GANGLION. R. I. Birks* and F. C. MacIntosh. Dept. of Physiology, McGill Univ., Montreal, Canada.

Cat superior cervical ganglia were perfused with eserine-containing solutions: the acetylcholine (ACh) released into the venous effluent during maximal preganglionic stimulation was assayed biologically, and at the end of the experiment the ganglion and its unperfused fellow were extracted for assay of bound ACh. The rate of ACh synthesis was calculated from these data. As previous workers have shown, ganglia stimulated at 20/sec during perfusion with Locke's solution cannot long maintain their high initial rate of ACh output; we find that they synthesize about 5×10^{-9} gm/min of ACh and lose about half their bound ACh during an hour's stimulation. Ganglia perfused with eserinized cat or human plasma, and similarly stimulated, release ACh at about the same rate initially, but in this case the output is maintained at a high steady level. Such ganglia synthesize more than 30×10^{-9} gm/min and double their bound ACh in an hour. Plasma dialysates support ACh release as well as plasma, and can be shown to contain a heat-labile factor necessary for optimal ACh synthesis. Unstimulated plasma-perfused ganglia also double their ACh in a hour although ACh release from them is undetectable. Synthesis is moderately depressed in ganglia perfused with CO_2-free or over-eserinized plasma, and is reduced by 90% when the hemicholinium base HC-3 (Schueler. J. Pharmacol. 115, 127, 1955) is present at 10^{-5} M concentration: this compound specifically inhibits ACh synthesis, and eventually blocks synaptic transmission, by interfering with a choline-transport mechanism. For frequencies below 20/sec, each maximal preganglionic volley releases about 5×10^{-11} gm of ACh from the plasma-perfused ganglion; with more rapid stimulation the output per volley varies inversely with the frequency. (Supported by the Defense Research Board of Canada.)

50. FUNCTIONAL DISTRIBUTION OF FLOW WITHIN THE KIDNEY. William D. Blake. Univ. of Oregon Med. School, Portland.

Experiments were conducted on dogs anesthetized with chloralose or sodium pentobarbital. Both ureters were cannulated with polyethylene tubing and urine samples collected separately from the 2 kidneys. A no. 20 needle was inserted into the left renal artery via the aorta under direct visualization (flank incision). After 2 control urine collection periods, sodium pentobarbital was infused into the left renal artery at the rate of 10 mg/min in 0.25 ml of 0.9% NaCl solution. Functions of the left kidney before and after pentobarbital infusion as well as functions of the right kidney were used for detecting changes. During pentobarbital infusion, urine flow and sodium excretion from the left kidney increased but glomerular filtration rate (creatinine clearance, Ccr) and glucose Tm, TmG, usually decreased. Ccr decreased proportionately less than TmG, hence the ratio Ccr/TmG increased. An identical response has been observed when one of a pair of arteries supplying a single kidney was occluded. The most likely explanations are believed to be that sodium pentobarbital and renal ischemia may 1) almost completely remove one segment of the nephron population from the renal circulation and increase glomerular filtration in the remaining perfused segment; 2) impair the tubular reabsorptive process for sodium and glucose and/or 3) redistribute flow away from a nephron population area having a relatively low rate of glomerular filtration per unit of tubular reabsorptive capacity to an area in which the nephrons have a relatively high glomerular filtration rate per unit of tubular reabsorptive capacity.

51. MEASUREMENT OF CO IN BLOOD AND PRODUCTION OF CO BY THE DEGRADATION OF HEMIN. W. S. Blakemore, G. D. Ludwig, R. E. Forster, and D. L. Drabkin (intr. by I. S. Ravdin). Harrison Dept. of Surgical Research, Johnson Research Fndn., Dept. of Medicine, School of Medicine, Depts. of Physiology-Pharmacology and Biochemistry, Univ. of Pennsylvania Grad. School of Medicine, Philadelphia.

An in vitro method for measuring low concentration of CO in blood has been developed using a) 10% H_2SO_4 or b) ferricyanide to release CO from hemoglobin and measuring the extracted CO in an infrared gas analyzer. The S.D. of a single estimation in the range 0.17-1.5 vol. % CO, was 0.05 vol. %. Analysis by the chemical method of Roughton and Root was not significantly different. Sjostrand using a "hopcalite" CO analyzer found that CO may be formed in these methods by the degradation of hemin. While unable to confirm Sjostrand's results, we found that in the conversion of crystalline hemin to verdohemochromogen CO was formed and identified as follows: infrared CO analysis, formation by HbCO identified spectrophotometrically, and oxidation to CO_2 by iodine pentoxide or palladium chloride. Experiments with C^{14} labeled hemin indicate that CO is formed by the coupled oxidation of the alpha methene bridge carbon. Further studies of the kinetics of the formation of verdohemochromogen show that the green pigment was formed prior to the release of CO, suggesting that the methene bridge was oxidized to a carbonyl group before the tetrapyrrole ring opens completely.

52. RENAL HEMODYNAMICS AND FUNCTION DURING HYPOTHERMIA. C. Blatteis and S. M. Horvath (intr. by W. W. Tuttle). Dept. of Physiology and Cardio-vascular Lab., State Univ. of Iowa, Iowa City.

Renal function studies were made in anesthetized dogs cooled by immersion in an ice bath. During

induction of hypothermia, rectal temperature dropping, 0.04°C/min the renal clearances of creatinine and PAH remained at near the control values until the rectal temperature had fallen approximately 3°C. This period was generally coincident with vigorous, generalized shivering and maximum oxygen utilization by the animals. As rectal temperature was lowered further, the glomerular filtration rate, effective renal plasma flow, and tubular excretion of PAH all decreased at about the same rate, i.e. 5% of control for each 1°C decrease in rectal temperature. The rectal temperature became stabilized at 23°C ± 2, these renal functions continued to decline. Above the changes observed were reflected by an initial increase in filtration fraction, which persisted or decreased subsequently, approaching the control value as hypothermia became well established. Tubular reabsorption of water remained at control levels throughout the experiment. The urine volume output in the hypothermic dogs was above control during induction of hypothermia, but fell below control levels when hypotheria was established.

53. ENCEPHALITIC ACTIVITY OF THE ETHER-SOLUBLE FRACTION OF FOLCH AND LEES. Lasca H. Bogdanove* and George Clark. Dept. of Physiology, Univ. of Buffalo, Buffalo, N. Y.

The ether soluble fraction (E) of the original Folch and Lees method for separation of proteolipids was prepared from horse spinal cord (HSC) and from horse peripheral nerve (HPN). An average yield of 4.5 gm could be obtained from 60 gm HSC but only an average of 1 gm could be extracted from 60 gm HPN. These extracts were incorporated into a Freund type emulsion and injected intradermally into the nuchal region of guinea pigs at a dosage level of 1 mg/animal. Control animals either received no injection or injections of adjuvants plus liver or adjuvants plus saline. Electrolytic lesions were placed 5 or more days after injection of the emulsions, and animals were sacrificed 25 or more days after the lesions were made. Portions of the brain, spinal cord and peripheral nerves (sciatic and brachial plexus) were studied histologically. In 5 of 8 animals injected with HSC-E there were found in the central nervous system (CNS) the typical changes of allergic meningoencephalomyelitis. In 2 of 8 animals injected with HPN-E there were found in the CNS the typical changes of allergic meningoencephalomyelitis. No pathological changes were seen in the peripheral nerves of any of the animals. (Supported by research grant B-825 from the Natl. Insts. of Health, PHS.)

54. EFFECT OF ELECTROLYTES ON DUAL CONTRACTILE RESPONSE OF THE ARTERY STRIP TO EPINEPHRINE. David F. Bohr, Donald C. Brodie* and Donald Cheu.* Dept. of Pharmacology and College of Pharmacy, Univ. of California, San Francisco, and Dept. of Physiology, Univ. of Michigan, Ann Arbor.

Spiral strips of rabbit aorta were mounted in Krebs solution containing calcium versenate, 10^{-5}. Isotonic contractions were recorded. Epinephrine, at a concentration causing about 1/3 maximum contraction, was added at 20-min intervals and allowed to remain 5 min. An increase in NaCl from 118-155 mM caused a decrease in the amplitude of contraction and an increase in the rate of relaxation. A decrease to 85 mM caused converse effects. Total osmolarity was kept constant by varying the sucrose content of the Krebs solution. Control experiments in which NaCl was kept constant and the sucrose alone altered showed the variations in relaxation rate, but not those in amplitude of contraction. An increase in KCl from 4.7-9.4 mM caused a marked increase in contraction amplitude while its reduction had the converse effect; neither change altered the rate of relaxation. Observed variations in the shape of the contraction curves induced by these electrolyte shifts requires the interpretation that epinephrine causes shortening by 2 differentiable processes; a fast process reaching its peak in 60-90 sec, which is little changed by the above electrolyte shifts, and a slower process, normally responsible for continued shortening throughout the 5-min observation period, which is eliminated by high NaCl or low KCl and potentiated by the converse of these changes. (Supported by Research Grant H-2431, Natl. Insts. of Health, PHS.)

55. VOMITING IN EXPERIMENTAL UREMIA. H. L. Borison, L. Hebertson,* R. Jensen,* J. Nelson* and M. Baker.* Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

In 12 normal dogs, bilateral nephrectomy was followed regularly by vomiting with an average latent period of 28 hr (range: 16-48). In each of 3 dogs with chronic transthoracic vagotomy, nephrectomy resulted in vomiting within 24 hr postoperatively. Of 12 chronic dogs with effective lesions of the medullary emetic chemoreceptor trigger zone (CT zone), as indicated by elevated emetic thresholds to apomorphine and/or lanatoside C, 2 dogs did not vomit following nephrectomy up to the time of death on the 5th and 6th postoperative day, respectively; the 10 remaining CT-zone ablated dogs vomited after nephrectomy with an average latent period of 85 hr (range: 54-147). In 4 chronic dogs with ineffective lesions of the CT zone, vomiting resulted from nephrectomy with an average latent period of 32 hr (range: 18-47). Guanidine, 75 mg/kg intravenously, evoked vomiting within 15 min in all of 5 normal dogs. The same dose produced an emetic response in only one of 9 chronic dogs with effective lesions of the CT zone. Post-nephrectomy treatment with morphine and with chlorpromazine failed to prolong the latent period of emesis beyond the normal limit. In cats, the latent period of vomiting after nephrectomy was essentially the same as in dogs. All of 3 CT-zone ablated cats failed to vomit during 3 days of survival following nephrectomy. (Supported by Grant No. B-491, Natl. Insts. of Health, PHS.)

56. INFLUENCE OF MAGNESIUM ION UPON TENSION DEVELOPMENT BY MUSCLE FIBER BUNDLES. William J. Bowen, Natl. Insts. of Health, Bethesda, Md.

Rates of isotonic shortening of myosin B threads and of glycerol-treated rabbit psoas fibers produced by ATP do not parallel the changes in rates of splitting ATP by myosin which are produced by varying the ionic milieu (Am. J. Physiol. 165:10, 1951; 169: 223, 1952; Biochim. et Biophys. Acta 18:83, 1955). The question arises whether such non-parallelism would exist if isometric rather than isotonic contraction were used. Tension development by psoas fibers has been studied with the aid of the Ranney myograph (J. Appl. Physiol. 6:513, 1954) under ionic conditions, the varying of which affects model shortening and splitting of ATP differently. In 6 solutions each containing 0.04 M KCl, 0.02 M tris (pH 7.5) and 0.001 M ATP and individually containing 0 M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 3 x 10^{-2} M $MgCl_2$, the mean isometric tensions developed were 12.65 ± 1.30, 19.97 ± 2.51, 24.03 ± 2.15, 29.89 ± 2.52, 32.85 ± 2.84 and 31.90 ± 3.47 gm/mm^2, respectively. The rates of splitting ATP by homogenized psoas fibers in the same medium

increased as [$MgCl_2$] was increased from $0.0\text{-}10^{-4}$ M and then decreased sharply as [$MgCl_2$] was increased to 0.03 M. In solutions containing only tris, ATP, and $MgCl_2$ as above, the tensions also increased and remained high, but the amounts of ATP split decreased sharply above 10^{-4}M $MgCl_2$. Increasing [KCl] in the presence of 0.001 M $MgCl_2$ does not decrease tension, except at 0.3 M KCl. The latter is thought to be correlated with the dissolution of myosin at concentrations of KCl $\geq$ 0.3 M.

57. CORRELATIONS BETWEEN ACTION POTENTIAL AND CONTRACTILE TENSION OF FROG VENTRICLE. Allan J. Brady (intr. by J. Walter Woodbury). Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.

Excised frog ventricles were perfused with Ringer's solutions of varied ionic composition. The entire ventricle was excited, approximately simultaneously, by passing rectangular current pulses radially outward through the wall. Intracellular potentials were recorded with a flexibly mounted ultramicroelectrode. Contractile tension was recorded with an RCA mechanoelectrical transducer connected to the apex of the ventricle. Temperature was maintained at 10°C and stimulus rate at 8/min. Contractile tension was several times normal in 50% of normal sodium solutions, and slightly below normal in 20% of normal sodium. Contractile tension decreased sharply in K-free solutions. A slope of approximately one was found for the curve relating the duration of membrane action potential and the peak of contractile tension in 50% and 20% of normal sodium solutions, in K-free solution, and during 4-fold changes in rate. Ratio of peak tension to rise time of tension increased uniformly with decreased extracellular sodium concentration. The close correlation between duration of action potential and maximum contractile tension suggests that the fiber membrane potential determines the duration of the active contractile state. There appears to be a qualitative correlation between rate of rise of tension and the slope of the second phase of repolarization of the action potential. (Aided by Grant B-82 from the Natl. Inst. of Neurological Disease and Blindness, PHS.)

58. DEPRESSION OF CENTRAL BLOOD VOLUME AND CARDIAC OUTPUT DURING POSITIVE PRESSURE BREATHING; RE-ELEVATION BY ARAMINE. E. Braunwald, W. L. Morgan, Jr.,* J. T. Binion* and S. J. Sarnoff. Lab. of Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

Effects of high levels of positive pressure breathing (PPB) on cardiac output, central blood volume and arterial pressure were investigated using a continuously recording cuvette densitometer for the application of the Hamilton dye-dilution technique. In 18 dogs subjected to an average mean airway pressure of 25 cm H_2O, cardiac output fell an average of 72%, central blood volume fell an average of 35% and mean femoral arterial pressure fell an average of 56%. Cardiac output and arterial pressure rose substantially in the 8 dogs to which Aramine was administered (0.2 mg/kg, i.v.) while PPB was maintained; central blood volume rose in 7 of these 8. In 3 of 4 dogs, Aramine administered prophylactically afforded significant protection against the circulatory depression subsequently induced by PPB. Circulatory depression during PPB results primarily from the interference with thoracic inflow imposed by elevated intrapleural pressures. As a consequence, peripheral blood volume increases at the expense of intrathoracic blood volume until venous pressure rises to that level which will overcome the intrathoracic impediment to flow. The in vitro and in vivo action of Aramine on veins, taken together with the above data, suggests that a significant part of the circulatory support provided by this agent is due to its effect in decreasing venous distensibility and thereby re-elevating intrathoracic blood volume. This may be thought of as an internal pressure suit.

59. PRESSURE-VOLUME RELATIONS IN THE QUIESCENT VENTRICLE. Gerhard A. Brecher and Abbott T. Kissen.* Dept. of Physiology, College of Medicine, Ohio State Univ., Columbus.

It has been suggested that intraventricular diastolic suction of the nearly empty ventricle is caused in part by elastic forces of the ventricular wall. The problem of the existence of such forces under static conditions in the nearly empty ventricle was studied by examining the intraventricular pressure-volume relationships in the quiescent, but still viable ventricle. In open-chest dogs the zero pressure reference level (atmospheric pressure) was established by filling the chest with saline and submerging the ventricle. Inflow and outflow tracts of the heart were occluded, the ventricular content manually expressed and the empty ventricles then filled by increments of saline. It was found that elastic forces of the ventricular walls produce a subatmospheric (negative transmural) pressure in an empty ventricle (right and left). As fluid is added to a ventricle the negative intraventricular transmural pressures become zero and finally positive. The relationship of volume to negative transmural pressure represents a negative limb in addition to the known positive limb of the ventricular pressure-volume curve. Both limbs form an S-shaped curve in which the least pressure-volume change occurs approximately at zero transmural pressure. It is concluded that under static conditions elastic forces tend to restore diastolic ventricular dimensions in the nearly empty ventricle. Whether these forces operate in the normally filling ventricle and contribute to its filling cannot be determined from these findings.

60. EFFECT OF RELAXIN, ESTRADIOL, AND PROGESTERONE ON WATER CONTENT OF THE UTERUS OF THE RAT. D. M. Brennan* and M. X. Zarrow. Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.

An increase in uterine water from 79.6% to 85.4% was obtained at 6 hr after treatment with 50 guinea pig units (GPU) of relaxin. The concentration fell to 83.3% at 12 hr, and 79.8% at 32 hr. Dry weight determinations failed to indicate any true growth of the uterus, as contrasted with the response seen following estradiol. A dose response curve was obtained for relaxin over a dosage range of 0.5-10 GPU. Further increase in dosage to 100 GPU failed to elicit a significantly greater response. The simultaneous administration of relaxin and estradiol caused a synergistic effect. A marked increase in the uterine water content was also obtained in the adrenalectomized rat following treatment with relaxin. In general the response curve for normal and adrenalectomized animals were similar, except that the slope was steeper in the adrenalectomized group. A minimal dose of 1.25 mg cortisone acetate completely prevented the increase in uterine water, while 1 mg partially blocked this effect, following the administration of 10 GPU relaxin. Treatment with 0.25 mg of progesterone caused a partial block of the uterine water increase

following relaxin injection. (Aided in part by a grant from the Ortho Research Fndn. and the Purdue Research Fndn.)

61. EFFECTIVE FRONTAL PLANE VECTORCARDIOGRAMS OF RANA PIPIENS. Daniel A. Brody and Ralph F. Morton.* Cardiovascular Lab., Dept. of Medicine, Univ. of Tennessee, Memphis.

Frontal plane vectorcardiograms of Rana pipiens were recorded by means of a cubical immersion-tank lead system. The sides of the tank are provided with averaging electrode systems which rather accurately integrate electrocardiographic potentials over the frog's body and present the resulting information to the vectorcardiograph as the effective electrical moment of the heart. Exhaustive physical tests with a 3-dimensional model show that heart vectors are projected on the respective lead axes of the system with ± 2% precision. Similar experiments with frogs show the tank system is in effect a physically isometric reference frame in which the vectorcardiogram is invariant except for the known variable of body orientation. Comparison with vectorcardiograms recorded by the tetrahedron system show that the latter are unreliable for accurate determination of heart vector orientation and frontal plane projections of the QRS-T angle. In general there is good topological agreement between vector loops recorded by the 2 methods, but occasionally the tetrahedral system records sub-loops which have no counterpart in the effective vectorcardiogram. Experiments with immersion media of various resistivities indicate that electrical inhomogeneity of the frog body produces small but definite distortion of the body surface electrocardiographic potentials. An extension of the technic permits determination of the electrical moment of the heart in its correct units of dimension. The magnitude of the maximum QRS vector was found to be on the order of 2-3 microampere-cm. (Supported by research grant H-1362(C3) of the Nat. Heart Inst., PHS.)

62. SUSTAINED CORTICAL POTENTIAL CHANGES INDUCED BY THALAMIC STIMULATION. J. M. Brookhart, A. Arduini,* M. Mancia* and G. Moruzzi.* Ist. di Fisiologia, Univ. di Pisa, Pisa, Italy.

Potential changes at the surface of the cerebral cortex of the cat have been studied using D.C. recording techniques. Etherized animals have been subjected to stereotaxically placed electrolytic destruction of entering trigeminal fibers, section of the upper cervical sensory nerves, and later curarized. Thalamic foci (VPL, VL) giving rise to 8/sec augmenting waves, or foci (Reu., Submed.) giving rise to 8/sec recruiting waves have been stimulated electrically at 40-60/sec. This higher frequency stimulation is accompanied by surface negative potential shifts of 500-2000 μv. Potential shifts induced by higher frequency stimulation are localized to the same areas from which the recruiting or augmenting responses are evoked or recorded, endure for the period of stimulation, and are abolished by local cortical injury. The potential shift accompanying midline thalamic stimulation rarely reaches the maximum amplitude of the negativity of the recruiting waves. The potential shift accompanying lateral thalamic stimulation often exceeds the maximum negativity of the augmenting waves. Curves of surface negativity vs. voltage are not the same for the potential shifts and the 8/sec waves. Intravenous Nembutal (20-30 mg/kg) reduces the potential shift without materially affecting the amplitude of the 8/sec negative waves. (Aided by Rockefeller Fndn. and Consiglio Nazionale delle Ricerche.)

63. MECHANICAL PROPERTIES OF THE STRETCH REFLEX. Arthur C. Brown (intr. by Allen M. Scher). Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.

The calcaneus tendon was freed from its insertion in a cat with a midcollicular decerebration. The tension developed by the muscles attached to the tendon (gastrocnemius and soleus) was measured as a function of the type of stretch applied to the tendon. If the length of the tendon was varied as length = L sin $2\pi ft$, L approximately 1 cm, the shape of the dynamic length-tension graph was independent of frequency over a range 0.2--5.0 cps. During the half cycle while the length was increasing (velocity positive), the tension was directly proportional to the stretch; in the half cycle during which the length was decreasing (velocity negative), the tension fell to a very low level. This pattern of response resulted in a large hysteresis loop whose area represents the work necessary to drive the system. If the sine motion was stopped suddenly and the length held constant, the tension approached a static value about two thirds of that during the velocity positive phase. If the sine motion was begun suddenly, the tension moved from the static to the dynamic curve in less than one-quarter cycle. The above phenomena resulted from the active reflex and did not represent passive properties of the muscle. This was shown by the fact that blocking the reflex by nerve action or decamethonium bromide reduced the tension during all the above maneuvers to about 10% of the maximum active level. It was concluded that the results are not consistent with linear models (e.g. elasticity and viscosity), but must be viewed in terms of a non-linear control system. (Aided by grants B-395 and H-2397 PHS.)

64. PREDICTION OF BLOOD VOLUME IN HEALTHY MEN. E. Brown, J. L. Hodges, Jr.,* R. Wennesland,* J. Hopper, Jr.,* K. G. Scott,* I. N. Tucker,* O. Guttentag* and B. Bradley.* Dept. of Medicine and Statistics and the Radioactivity Ctr., Univ. of California, San Francisco and Berkeley.

Data from 201 Cr^{51} determinations in healthy prison inmates show that red cell volume (VRBC) can be predicted more accurately when related to height and weight combined than to either measure alone. (S.D. about regression line of VRBC related to height = 0.248 l., related to weight = 0.197 l., and S.D. about regression plane of VRBC related to height and weight = 0.191 l.). Graphically, the 3-dimensional system can be represented by a surface whose axes are height and weight, with contour lines representing predicted volumes. Using this chart, individual deviations from the weight:height relations of the "normal" population are revealed, whereas these are concealed when surface area is calculated from DuBois' formula. Analysis of residuals shows that consideration of somatotype and habits of physical activity improves prediction modestly. Plasma volume (calculated from hematocrit) is relatively more dependent on height, and VRBC on weight. The volume of blood per unit body size appears not to decrease with increasing body size.

65. ARTERIO-VENOUS POTASSIUM DIFFERENCE ACROSS THE HEART IN SEVERE ACIDOSIS. E. B. Brown, Jr. Dept. of Physiology, Univ. of Minnesota, Minneapolis.

In previous work we have observed an increase in plasma potassium concentration in dogs during 4 hr of breathing 30%-40% CO_2-O_2 mixtures. On returning the animals to air breathing a further and much more rapid increase in plasma potassium level takes place. The highest concentration reached is often double control level or higher. In the present study

simultaneous blood samples have been drawn from the coronary sinus and aorta before, during, and after dogs have breathed 30% CO_2 - 70% O_2 for 2 hr followed by 40% CO_2 - 60% O_2 for an additional 2 hr. Plasma potassium concentration was determined on a protein free filtrate by flame photometry. There was no difference in potassium concentration between the control arterial and venous samples. As plasma potassium level increased during respiratory acidosis the heart appeared to be taking up potassium from the blood. With the rapid fall in CO_2 tension and H^+ concentration following return to air breathing, and while plasma potassium level was still rising, potassium concentration in coronary sinus blood was regularly above that in arterial blood, indicating that the heart gives up potassium during the critical post-hypercapnic period.

66. AVOIDANCE AND APPROACH LEARNING MOTIVATED BY STIMULATION OF IDENTICAL HYPOTHALAMIC LOCI. George W. Brown and Bertram D. Cohen.* General Med. Research Lab., VA Hosp., Iowa City, Iowa.

The present series of experiments test the hypothesis that a hypothalamic stimulus may act as an energizing, non-associative, drive-arousing operation to produce both avoidance and approach learning in cats. A group of cats with stimulating electrodes implanted in the lateral hypothalamus have been subjected to 2 types of experimental procedures. In the first procedure the animals were given an opportunity to learn to avoid a hypothalamic stimulus which produced a typical "hypothalamic rage" response. The second procedure allowed the same animals to learn to approach an area where a similar hypothalamic stimulus was given. In both procedures similar stimuli were delivered through identical electrodes, yet each animal learned the appropriate avoidance or approach response depending upon the experimental condition. Thus it would appear that the concept of "reward centers" or "aversive centers" applied to the area of the lateral hypothalamus in cats is meaningless.

67. INFLUENCE OF ALTERATION OF STRUCTURE ON STEROID METABOLISM IN TISSUE SLICES. J. H. U. Brown and Atalante Anason.* Dept. of Physiology, Emory Univ., Ga.

The metabolism of 16 steroids have been investigated by several reactions. The steroids were added to rat liver slices with due precautions and the alterations in rate of metabolism followed by chemical tests. The Porter-Silber reaction (P-S) was used to estimate loss of the side chain, the BTZ reaction to indicate loss of a 17-hydroxyl (when subtracted from the P-S), the E-240 to estimate changes in the A-ring, the Gornall to estimate changes in the 11 position when combined with the P-S and E-240, and the chromotrophic acid reaction to estimate changes in formaldehydic steroids. In general, alteration of the A-ring by addition of a double bond or a methyl group appeared to inhibit the rate of metabolism of this grouping. The rate of metabolism of the side chain appeared to be little affected by changes in other parts of the molecule. Prednisolone appeared to be the only structure in which significant changes in the 17-hydroxyl position occurred. The presence of a 9α fluoride did not appreciably alter the rate of metabolism of either the A-ring or the side chain. Metabolism of other compounds will be discussed. (Supported by PHS Grant C-2339.)

68. INTRARETINAL ERG OF THE UNOPENED CAT EYE. Kenneth T. Brown and Torsten N. Wiesel (intr. by Stephen W. Kuffler). Wilmer Inst., Johns Hopkins Med. School, Baltimore, Md.

A technique has been developed to use micropipette electrodes in the retina of the unopened cat eye, and this technique has been applied to analysis of the ERG. When the electrode is located in the receptor layer, as indicated by recording depth from the retinal surface, the ERG consists of an a-wave, b-wave, c-wave, and what appears to be a separate negative DC component. If stimulus intensity is reduced, or if the retina is light adapted, the DC component may be seen in isolation. Thus Granit's analysis must be extended to include the separate negative DC component, at least in the case of the cat. All 4 components have been tentatively identified with their counterparts in standard external recording. When penetrating the retina, the b-wave reverses polarity with respect to a vitreous lead at about 30% of the total retinal depth, followed slightly deeper by a reversal in polarity of the DC component. Then these components increase in magnitude to a maximum near the external limiting membrane, and drop off sharply in the presumed region of Bruch's membrane. The a-wave is found only within a depth range not exceeding about 60 micra, at a location just short of Bruch's membrane, and it is reversed in polarity. The c-wave does not reverse polarity and it goes through a maximum in the same region where the a-wave is found.

69. SMOKING AND BODY COMPOSITION. Josef Brožek and Ancel Keys. Lab. of Physiol. Hygiene, Univ. of Minnesota, Minneapolis.

Data on smoking habits and physique were analyzed for samples of men drawn from several occupational groups. The criteria of physique included 1) relative body weight, 2) direct body measurements indicative of fatness and muscularity, 3) soft-tissue x-rays of the limbs and 4) determinations of body density as an indicator of total body fat. In middle-aged business and professional men no significant differences were noted between the "Never" and "Heavy" (more than 1 pack of cigarettes a day) smoker groups for relative weight, arm diameter corrected for subcutaneous fat (as a measure of muscularity), or body density, when either cigarettes alone or all forms of tobacco smoking were considered. The sum of 3 skin-folds was somewhat larger in the "Never" group compared with heavy cigarette smokers but there was no difference when all heavy smokers were considered. In railroad clerks and switchmen there was no important difference in relative body weight between "Never" and any category of smoking. Similarly, no significant difference was seen between "Never" and "Heavy" cigarette smokers in regard to the ratio of subcutaneous fat thickness to bone diameter in the extremities. The ratio of muscle width to bone width was higher in the "Never" group for each of 3 sites (calf, thigh, upper arm). In a longitudinal study, 17 men who stopped smoking, without a superimposed reducing diet, were followed for 5 years. The mean weights for 2 years prior to stopping were 75.3 and 75.8 kg and increased to 78.4 and 79.3 in the subsequent 2 years. The increase was statistically highly significant.

70. LIVER COENZYME A AND TOTAL FATTY ACIDS IN TURTLES UNDER VARYING ENVIRONMENTAL CONDITIONS. Stanley J. Brumleve* and X. J. Musacchia. Dept. of Biology, St. Louis Univ., St. Louis, Mo.

Experiments were designed to provide additional information concerning liver fatty acids and coenzyme A concentration in turtles. Four groups of animals were used. One group was maintained under laboratory

conditions for 8 wk, a 2nd group was fasted at room temperature for 8 wk, a 3rd group was maintained between 0°C and +5°C for 8 wk, while the 4th group, "wild" animals, was sacrificed within a few days after capture. Total liver fatty acids were determined and coenzyme A was assayed. The "wild" animals had the highest coenzyme A concentration, 49.6±2.0 units, and the highest total fatty acids, 5.47±0.86 mg %. Starved animals had the least coenzyme A concentration, 29.2±2.6 units and total fatty acids were 2.81±0.44 mg %. Laboratory animals and cold-exposed animals had approximately the same coenzyme A concentration, and comparable quantities of total fatty acids. Coenzyme A in laboratory animals was 39.0±1.5 units and total fatty acids 3.80±0.64 mg % while coenzyme A and total fatty acids values were 35.5±1.6 units and 4.09±0.21 mg % for cold-exposed animals.

71. CONTRACTILITY OF DYSTROPHIC MOUSE MUSCLE. Manfred Brust* and Alexander Sandow. Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City. Gastrocnemii from dystrophic and normal mice (Jackson Memorial Lab. strains) were studied at 15°C which permits prolonged, uniform maintenance of standard responses. Results are averages from muscles of generally 10-12 diseased or normal animals. Normal muscles (86.6 mg) produce maximal twitch and tetanus tensions of, respectively, 23.9 and 53.1 gm; dystrophic muscles (35.6 mg) correspondingly yield 3.3 and 14.6 gm. Thus, dystrophic muscles are much weaker than normal, involve a smaller twitch to tetanus ratio, and have a smaller tension output per gm muscle (especially in twitch). Although the contraction period time is identical for both muscle types (about 60 msec), the relaxation period lasts 3X longer in dystrophic ones (676 as against normal of 224 msec). In responses to 2 maximal shocks, with interval varying from 10-200 msec, the tension increment due to the second shock relative to the output evoked by the first alone is always greater for the dystrophic muscle. In isotonic experiments at 25°C, treatment with 10^{-4} M iodoacetate causes dystrophic muscles to develop passive rigor more slowly; and in activity rigor, they develop less maximal rigor shortening than the normal, but they attain it and then relax from it more quickly. The results suggest that muscular dystrophy involves certain possible quantitative and qualitative derangements of the contractile system, the greatly extended relaxation period especially indicating disturbance of mechanisms (relaxation enzyme systems?) upon which relaxation may depend. (Supported by Muscular Dystrophy Assocs. of America, and Office of Naval Research.)

72. INTRACELLULAR RECORDING FROM END PLATE REGIONS OF THE RAT M. GRACILIS ANTICUS IN SITU. S. H. Bryant (intr. by G. H. Acheson). Dept. of Pharmacology, Univ. of Cincinnati, Cincinnati, Ohio.

A circulated muscle in situ offers many advantages over an excised muscle for the study of neuromuscular phenomena. In these studies the end plate region at the pelvic end of the muscle was utilized. End plate regions were localized by visual mapping of the nerve field near the thin posterior border of the muscle. Since the end plates per se were not visualized, after impalement with microelectrodes these areas were removed and stained with Schiff's reagent. These anatomical preparations show the distribution of nerve endings. The probable range of deviation of a microelectrode from and end plate center was usually less than 500 μ. The resting and action potentials in the rat gracilis anticus were similar to those reported in other vertebrate skeletal muscles. An outstanding feature was the relatively small end plate depolarization required to elicit an action potential. Furthermore the muscle action potential was less distorted by end plate changes than it is in the frog sartorius. With locally applied neuromuscular blocking agents (either depolarizing or non-depolarizing) the critical depolarization during the development of block was usually less than 10 mv.

73. CHOLESTEROL BIOSYNTHESIS IN LIVER CELL FRACTIONS FROM X-IRRADIATED AND FASTING RATS. Nancy L. R. Bucher, Alden V. Loud* and Kathleen McGarrahan.* Huntington Mem. Labs., Harvard Univ., Massachusetts Gen. Hosp., Boston.

The specific activity of cholesterol from livers of fasting x-irradiated rats injected with C^{14}-labeled acetate is 20-30 times greater than that of fasting controls (Gould et al. Fed. Proc. 15:264, 1956). Experiments were performed in vitro with slices and cell-free preparations of livers from control and x-irradiated rats (2400 r, whole body) fasting from treatment until sacrifice 24-48 hr later. Slices from irradiated rats incorporated 10 times as much C^{14}-acetate into cholesterol as controls. Cell-free preparations (J. Biol. Chem. 222:1, 1956) of controls were considerably less active than slices from the same liver, whereas those of irradiated animals were equal to or higher than the corresponding slices. Addition of substrate (hexose diphosphate - HDP) did not abolish this effect. Cell-free preparations from irradiated livers exhibited 100 times the activity of the fasting control preparations even in the presence of HDP. That sufficient HDP was provided was demonstrated in highly active preparations from irradiated rats: activity was abolished by elimination of endogenous glycogen with salivary amylase and entirely restored by addition of HDP. Mixed preparations containing irradiated microsomes plus control supernatant, or control microsomes plus irradiated supernatant showed considerably less activity than irradiated microsomes plus irradiated supernatant. These findings suggest a deficiency other than substrate involving both the microsomal and soluble cell constituents in the livers of fasting rats. The diminished activity in fasting controls is absent in irradiated rats which actually exhibit activity several times that of normal fed animals.

74. WORK OF ISOLATED MAMMALIAN LEFT VENTRICLE. N. M. Buckley and N. Zeig (intr. by H. D. Lauson). Albert Einstein College of Medicine, New York City.

The work performed by the mammalian left ventricle has been measured under a variety of conditions in the functionally isolated left ventricle of the dog heart prepared by a technic previously described (Circulation Res. 4:148, 1956). The right ventricle is kept empty and performs no external work. Left ventricular pressure and left ventricular volume changes are displayed on a dual trace oscilloscope in such a manner as to plot these 2 variables against one another throughout the cardiac cycle. The area of the calibrated records of these pressure-volume loops is measured by planimeter, and equated to the total external work of the left ventricle. Work has been calculated over a range of hydrostatic filling pressures when aortic pressure and heart rate have been controlled, in isolated left ventricle preparations

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freshly made. The preparations are then stressed by setting the artificial peripheral resistance at a high level. The work done in response to subsequent changes in hydrostatic filling pressure at the new high aortic pressure has been calculated during the development of left ventricular failure. Calculations of ventricular compliance ($\Delta V/\Delta P$) of the ventricle wall in diastole have been made for each work period. The role of the compliance of the ventricle wall in diastole as a determinant of ventricular work will be discussed in relationship to ventricular failure.

75. PHYSICAL FACTORS OF THE SKIN BARRIER LAYER AND WATER DIFFUSION INTO HUMAN SKIN. Konrad J. K. Buettner and George F. Odland.* Depts. of Meteorology, Physiology, and Anatomy, Univ. of Washington, Seattle.

Experiments have demonstrated substantial flows of water and water vapor through human skin in either direction. This transfer seems osmotic in nature. It is controlled by a hypothetical intracutaneous concentrating mechanism and by a diffusion barrier or resistance layer above the concentrating layer. The concentrating mechanism seems to be stronger in people suffering from water retention syndromes. The diffusion barrier has now been isolated using the stripping technique of Alexander Szakall; this layer is called stratum corneum conjunctum. The measured water and water vapor resistance of the barrier layer in vitro coincides nearly with the average resistance measured on 500 people in vivo. Consequently this layer contains nearly all diffusion resistance of the skin. Anatomical features of the barrier from electronmicrographs, its composition, electrical resistance, etc. will be discussed. The stripping technique will be demonstrated.

76. ANTAGONISTIC EFFECT OF ACETAZOLAMIDE AND DESOXYCORTICOSTERONE ON RENAL ELECTROLYTE EXCRETION IN THE NORMAL AND ADRENALECTOMIZED RAT. A. Burks,* J. Schiller and M. Kenney.* Depts. of Neuro-Psychiatry and Research, VA Hosp., New York City.

Groups of 6-12 adult female albino rats were kept 3 in a cage on a diet containing 0.338 gm % of K^+ and 0.0059 gm % of $Na.^+$ The 24-hr urinary excretion of electrolytes averages: Na^+ 0.522, K^+ 76.2 and Cl^- 4.27 μEq/cc. The intramuscular injection of 3-4 mg of acetazolamide resulted in the following output: Na^+ 106.16, K^+ 171.9, Cl^- 21.15 μEq/cc. As a result of the administration of 1 mg daily of desoxycorticosterone for 1 wk followed by acetazolamide urinary Na^+ changed from 0.68-53.8 μEq/cc, K^+ from 256.6-166 μEq/cc and Cl^- from 6.03-15.26 μEq/cc. With 3 mg daily of desoxycorticosterone followed by acetazolamide the output in Na^+ was 53.3, K^+ 187 and Cl^- 12.9 μEq/cc. Adrenalectomized rats on the same diet plus 1% Na Cl solution averaged urinary Na^+ 0.134, K^+ 0.058 and Cl^- 0.154 μEq/cc. Acetazolamide administration increased the output of Na^+ to 0.218 and K^+ to 0.117 μEq/cc and decreased the Cl^- to 0.079 μEq/cc. One week of 3 mg daily of desoxycorticosterone prior to the injection of acetazolamide reduced the output of Na^+ to 0.18, K^+ to 0.075 and Cl^- to 0.01 μEq/cc. NaCl solution was replaced by tap water 8 hr prior to the carbonic anhydrase inhibitor. In all experiments the output of cations was greatly increased in the first 4-hr period when the urinary pH reached values of 8.5 and over as against an average pH of 6 at the end of the following 20 hr. Desoxycorticosterone offers partial protection against the loss of Na^+ in both normal and adrenalectomized animals.

77. INFLUENCE OF HEAT ACCLIMATIZATION AND PHYSICAL CONDITIONING ON WORK PERFORMANCE AFTER DEHYDRATION. E. R. Buskirk,* P. F. Iampietro,* W. P. Beetham, Jr.,* and D. E. Bass. Environmental Protection Research Div., Quartermaster Research and Development Ctr., Natick, Mass.

Dehydration limits man's ability to work, largely through impaired cardiovascular function. Both physical conditioning and heat acclimatization appear to be associated with improvement in cardiovascular function during work. The present study was designed to determine whether the impaired ability to perform work during dehydration might be ameliorated by previous physical conditioning and/or heat acclimatization. Three groups of 5 men each were dehydrated overnight in the heat (115°F), on 2 occasions (D_1 and D_1), by approximately 5.5% of their starting body weight. During the 3-wk period between D_1 and D_2, 1 group (AC) was acclimatized to heat and physically conditioned; a 2nd group (C) was conditioned; the 3rd group (S) was sedentary. The ability to work after dehydration was assessed by the following criteria: "maximal" oxygen consumption ($\dot{V}O_2$), pulse rate (P) and rectal temperature (T_r), made at appropriate intervals. Dehydration produced a decrement in $\dot{V}O_2$ equal for all groups at D_1 and D_2. $\dot{V}O_2$ increased with conditioning in groups AC and C. Thus, $\dot{V}O_2$ at D_2 was higher than at D_1, for groups AC and C. Walking P and recovery P after work were lower at D_2 than at D_1, in groups AC and C, but not in group S. The elevation in T_r with walking and running was the same at D_1 and D_2. Physical conditioning enhanced work performance during dehydration, whereas acclimatization to heat did not appear to supplement this effect.

78. BREAKING POINTS OF TWO BREATH HOLDS SEPARATED BY A SINGLE INSPIRATION. S. M. Cain (intr. by F. N. Craig). Applied Physiology Branch, Chem. Warfare Labs., Army Chem. Ctr., Md.

Alveolar gas tensions were measured during 6 trails for each of 3 men at normal resting level, at the end of a maximum inspiration, at the breaking point of a first breath hold, at the end of a single gasp, and at the breaking point of a second breath hold. The volume of the inspirations preceding each breath hold as well as the breath-holding times were also measured. The first breath-holding time was substantially longer than the second. When Gray's chemical ventilation equation was used, the equivalent ventilation ratio at the first breaking point was significantly lower than that of the second breaking point. The value of the latter agreed with those reported elsewhere. It was concluded that other factors in addition to the chemical stimuli may affect the breaking point of breath holding and that the longer time of the first breath hold allowed these additional factors to lower the man's tolerance to chemical stimuli at the first breaking point.

79. SUSPECTED CIRCULATING "RADIOTOXINS" IN ADRENALECTOMIZED IRRADIATED RATS. R. D. Campo,* V. P. Bond* and E. P. Cronkite. St. John's Univ. Grad. School and Med. Dept., Brookhaven Natl. Lab., Upton, New York.

Interest in the abscopal effects of irradiation has been intensified since Edelmann (1955) presented evidence indicating the existence of a toxic substance(s) in the blood of irradiated adrenalectomized (ADX) rats. Subsequent experiments along lines similar to those outlined by Edelmann have been made in this laboratory. Thirty ADX female rats weighing 60-100 gm

were given 4.0 ml of serum intraperitoneally from 80 ADX male and female donor rats which had been irradiated 24 hr previously with 1000 r Co^{60} gamma irradiation. Donor rats were ADX'd 24 hr prior to gamma or x-irradiation and maintained on saline until sacrifice. Recipients were ADX'd 24 hr prior to injection and maintained on saline until injection time; thereafter they were put on plain tap water. Survival time and ultimate mortality of animals in the treated group was identical with that of 30 control ADX rats which had received serum from non-irradiated ADX rats, and with that of 30 non-injected ADX controls. Ninety-five ADX female rats weighing 40-60 gm received intravenously 0.5 ml of serum from x-irradiated ADX rats (1000 r). The survival pattern of this group was similar to that of 88 ADX control rats which received 0.5 ml of serum from non-irradiated ADX donors. Sixty-nine ADX non-injected rats showed a slightly greater survival rate not statistically significant. In other experiments ADX mature female mice injected intravenously with 0.6 ml of serum from ADX x-irradiated rats (1000 r) showed no increase in mortality. While the results of these experiments do not support the concept of a circulating "radiotoxin" in the blood of irradiated animals, they do not preclude the possibility that such a toxic substance may exist. (Supported by the U. S. Atomic Energy Comm.)

80. BIPHASIC PATTERN IN OPPOSITE "ON AND OFF" CO_2 EFFECTS ON METRAZOL SEIZURES. Charles R. Carey and K. E. Schaefer (intr. by Orr Reynolds). U. S. Naval Med. Research Lab., U. S. Naval Submarine Base, New London, Conn.

Exposure to 30% CO_2 in air or oxygen for 10 min produces a 33% increase in metrazol induced seizures in male albino rats of the Wistar-Hisaw strain. After removal of CO_2 no seizures were observed. One-hr exposure lowers seizure activity 35% below the control level. Transition from CO_2 to air results in 35% increase in the number of fits. At 2 hr the depression of seizure activity is lessened and the "rebound" effect is slightly lowered. During the stimulatory phase (10 min on 30% CO_2) the tonic flexor and extensor components of the seizures are more intense while during the depressive phase (1--2 hr on 30% CO_2) they are shortened and less intense and the clonic seizure prolonged. The pattern of the clonicotonic seizure occurring during CO_2 exposure and after removal of CO_2 does not appear to differ. Similar observations were made under the corresponding stimulatory and depressive phase of exposure to 10% CO_2 in air on the metrazol induced convulsion.

81. EFFECT [illegible] CHANGE IN ERYTHROCYTIC SIZE AND SHA[illegible] ON RATE OF NITRIC OXIDE UPTAKE. Elizabeth [illegible]elsen* and Julius H. Comroe, Jr. Dept. of Physiology and Pharmacology, Univ. of Pennsylvania Grad. School of Medicine, Philadelphia.

The overall rate of uptake of certain gases by red blood cell suspensions can be estimated by use of Forster's photoelectric modification of the Hartridge-Roughton rapid reaction apparatus: the rate of formation of O_2Hb, COHb, or NOHb can be measured at various points along an observation tube after jet-mixing of a deoxygenated erythrocyte suspension (1:40 dilution) with saline containing a known concentration of reactant gas. In these studies the reactant gas chosen was nitric oxide because the chemical combination of nitric oxide with hemoglobin is so rapid it cannot contribute significantly to the time involved in overall gas uptake by erythrocytes. Consequently, the limitations to rate of NOHb formation are the rate of nitric oxide transfer across the erythrocytic membrane, and/or intraerythrocytic diffusion. The rate of nitric oxide uptake by human erythrocytes at 38°C was measured using a) biconcave discs suspended in 0.9% phosphate buffered saline, b) the same cells sphered by suspension in 0.6% saline (mean corpuscular volume, MCV, increased by 28%; mean corpuscular hemoglobin concentration, MCHC, decreased to 25%), and c) the same cells crenated in 2 - 3% saline (MCV decreased by 32%; MCHC increased to 50%). There was no significant difference in rate of NOHb formation between a and b, but the cells in c showed a significant decrease in each of 10 experiments.

82. METABOLISM OF IMMATURE NERVE FIBERS IN THE DEVELOPING CHICK. F. G. Carpenter. Dept. of Physiology, Cornell Univ. Med. College, New York City.

Resting respiration of peripheral nerve trunks dissected from adult rats and chickens is usually between 15-20 μl. O_2/100 mg wet wt/hr, measured by the micro Warburg or by the Fenn differential volume micro-respirometer. The resting oxidative metabolism of nerves from rats and chickens of about 8 wk of age is roughly twice that of the adult. At hatching time when myelination and node formation in the chick is rather extensive, the Q_{O_2} of sciatic nerve trunks amounts to 75-85 μl. O_2/100 mg/hr and at day 12 when no myelination is apparent, the respiration rate of nerve varies between 125-150 μl. O_2/100 mg/hr. Pyruvate metabolism, as measured by its disappearance from media suspending the nerves, likewise diminishes in rate from the fetal to the adult stages. As early as day 10, chick sciatic nerves display electrical excitability and impulse propagation which progressively increases in velocity with the onset of myelination until the adult form is attained. It is believed that the energy requirements of the conducting elements and Schwann cells is high relative to the supporting elements such as myelin and connective tissue. Yet the rather low metabolism of adult nerve trunks relative to fetal nerve cannot be entirely explained by the subsequent appearance of lipids and epineurium during maturation and growth.

83. USE OF DPNH AS COENZYME FOR GLUTATHIONE REDUCTASE OF HEMOLYZATES. Paul Carson, Stanley Schrier and C. L. Flanagan (intr. by Alf S. Alving). Univ. of Chicago, Chicago, Ill.

Glutathione reductase catalyzes the reduction of oxidized glutathione. In many biological systems TPNH serves as specific coenzyme. DPNH can replace TPNH in hemolyzates of both normal and primaquine-sensitive human erythrocytes after dialysis against either phosphate or glycylglycine buffer (Science, 124:484, 1956). In a final volume of 7 ml containing 2.5 ml of hemolyzate and stoichiometric amounts of TPNH, 1.4×10^{-6} M of oxidized glutathione was almost completely reduced in 15 min at 37°C, pH 7.4, with or without added EDTA. When DPNH was used approximately 80% of oxidized glutathione was reduced without EDTA, 100% with EDTA. The DPNH used had no TPNH as measured by enzymatic assay with yeast glucose-6-phosphate dehydrogenase, which contained a high glutathione reductase activity specific for TPNH. The reductase reaction was stopped by addition of 5 ml of 1.7% metaphosphoric acid. To determine if DPNH had been converted to TPNH during reaction, the DPN formed was assayed in neutralized metaphosphoric acid filtrate with DPN-specific alcohol dehydrogenase at pH 8.5. Over 90% of the DPNH was thus accounted for.

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In the same filtrate no TPN was found by assay with TPN-specific yeast glucose-6-phosphate dehydrogenase at pH 7.4. After using TPNH, assay of neutralized filtrates showed no DPN and accounted for over 90% of the TPNH. (Army Med. Research Project, Contract number DA-49-007-MD-20.)

84. A METHOD FOR DETERMINING PULMONARY VENTILATION EFFICIENCY. Robert L. Carter,* Earl T. Carter* and Fred A. Hitchcock. Lab. of Aviation Physiology, Ohio State Univ., Columbus.

If during a standard nitrogen washout the expired gas is collected and continuously analyzed by means of a nitrogen meter it is possible to determine the functional residual capacity (FRC), the anatomical dead space (ADS), the tidal volume (TV), the resting alveolar volume (AV), and the concentration of nitrogen in the alveolar air at the end of any number of respiratory cycles ($(F_{A_N})_n$). Assuming homogeneous distribution, complete mixing and total DS washout, the theoretical ($(F_{A_N})_n$) will be $(F_{A_N})_I X^n + F_{CYL_N} Y (X^n - 1)/(X - 1)$ where $(F_{A_N})_I$ is the concentration of alveolar nitrogen prior to the start of the washout, "X" is (AV + DS)/(AV + TV), "Y" is (TV - DS)/(AV + TV) where DS is ADS plus instrument dead space, and F_{CYL_N} is the concentration of nitrogen in the gas used for the washout. Comparison of actually observed nitrogen washout with a washout predicted from the above principles provides a measure of ventilatory efficiency. Therefore, ventilation efficiency can be expressed as 100 (theoretical $(F_{A_N})_n$)/(actual $(F_{A_N})_n$ where the actual $(F_{A_N})_n$ is measured with the nitrogen meter and the theoretical $(F_{A_N})_n$ is computed using the above formula, after changing $(F_{A_N})_I$ to $(F_{A_N})_{n-1}$ and "n" to 1. Furthermore, the area between the predicted and the actual washout curves will be an index of the ventilation efficiency. It is understood that corrections for nitrogen excretion will have to be made in the above calculations.

85. NEUROLOGICAL SEQUELAE OF EXPERIMENTAL ANOXIA IN NEWBORN DOGS. S. Cassin and A. R. Fregly (intr. by G. R. Seaman). Dept. of Physiology, Univ. of Texas Med. School, Galveston.

Newborn dogs, all less than 24 hr old, were subjected to a pure nitrogen environment for times varying from 11-81 min. Five to 12 min after the last breath had occurred, artificial respiration (with pure oxygen and intermittent positive pressure) was instituted. The times of recovery varied considerably; one extreme case showed respiratory recovery 18 min after apnea. All anoxic animals demonstrated some degree of central nervous system insult, lasting 5-48 hr. Failure to suckle, opisthotonous, front limb extension, hind limb flexion and hypoesthesia were frequently noted. However, 1-6 months after the anoxic episode, no gross motor or behavioral aberrations were observed. Nevertheless, the same animals, subjected to psychological tests consisting of problem solving (open field mazes) and auditory discrimination (shuttle box), showed subtle behavioral differences.

86. COMPARISON OF CHLORIDE SPACE WITH OTHER EXTRACELLULAR SPACE ESTIMATES. W. O. Caster* and W. D. Armstrong. Dept. of Physiological Chemistry, Univ. of Minnesota, Minneapolis.

Sodium, chloride, thiocyanate and sucrose spaces have been estimated in each of the major tissues of the body and in the gastrointestinal contents. From these and tissue weight data, the total body sodium, chloride, thiocyanate and sucrose spaces have been estimated and compared with values obtained by dilution methods. Over 95% of the injected thiocyanate was recovered. Sucrose space values were made more uncertain by high and variable tissue blanks. With certain exceptions, the different space estimates were in reasonable agreement in all tissues. Following total body x-irradiation, the total body sucrose, raffinose and thiocyanate spaces were well correlated with each other but were quite different from the chloride space changes. When chloride and thiocyanate space changes in the different tissues were considered, a similar discrepancy was found. In the case of heart muscle where the plasma volume constitutes roughly 25% of the extracellular space, there was an excellent correlation between thiocyanate and Evans Blue space changes following irradiation but a poor correlation between chloride and Evans Blue space data. Since plasma and interstitial fluid are the tissue components with a high water content, one might expect changes in extracellular fluid volume to be correlated with the percentage of water in a tissue. In the heart, there was a good correlation between percentage tissue water and thiocyanate space but not between percentage water and chloride space.

87. REVERSAL BY CARDIOACTIVE AGENTS OF CO_2-INDUCED FAILURE OF THE HEART LUNG PREPARATION. H. Mead Cavert and G. G. Nahas.* Dept. of Physiology, Univ. of Minnesota, Minneapolis.

Acute myocardial failure is observed when the denervated heart-lung preparation (HLP) is rendered acidotic by increasing above 5% the CO_2 concentration in a respiratory gas mixture of CO_2 and O_2. Evidence of this failing state, described elsewhere (Nahas and Cavert, Fed. Proc., this volume), includes increased vena caval pressure, decreased aortic pressure, diminished cardiac output against a constant resistance and acute cardiac dilatation. After production of acidotic failure by administration of 15% CO_2 for 3-5 min (arterial pH of 7.1), values for these variables (with the exception of pH) can be returned to approximately control levels by injecting 4 μg/min of epinephrine or nor-epinephrine. Furthermore, the signs of CO_2 failure do not appear if the epinephrine infusion is begun concomitant with 15% CO_2 ventilation. Reversal of CO_2-induced failure is also observed within 5-10 min after injection of 0.1 mg of acetyl strophanthidin. Required dosages of the drugs vary with the alveolar CO_2 concentration and therefore with the severity and rapidity of acidotic failure. Since the mechanism of this failure remains undefined, its reveral by a sympathomimetic amine or by a digitalis analogue may be postulated as, alternatively, a non-specific net effect of opposing myocardial agents or a specific antagonism of CO_2-induced failure.

88. HEAT NARCOSIS AND ITS EFFECT ON RETENTION OF A LEARNED BEHAVIOR IN THE GOLDFISH. Jean A. Cerf and Leon S. Otis (intr. by Clara M. Szego). Neuropsychiatric Inst., Univ. of Illinois, Chicago.

A paralytic state is produced in the goldfish at a relatively low temperature (36.5 - 37°C). The slow electrical potentials of the vagal lobe (Adrian and Buytendijk. J. Physiol. 71:121, 1931) were recorded during heat narcosis and recovery. A reversible block of this spontaneous activity was demonstrated, suggesting similar electrophysiological correlates in other parts of the brain. This possibility has led to the investigation of eventual effects of heat narcosis on memory function. Goldfish were partially

conditioned (10 trials) in an avoidance situation to perform a positive response toward a shifting light in a 2-compartment tank. A single, brief, narcotizing heat treatment followed the training session at a chosen interval (0, 15, 60 min or 4 hr) for different groups of 15-19 subjects. Controls were run untreated, or submitted to a sub-narcotic temperature elevation. Overnight retention was measured by the savings in relearning the conditioned response. Criterion (5 consecutive correct responses in 10 trials) was met by 10.5%, 13.3%, 35.3% and 56.2% of the subjects paralyzed immediately, 15 min, 60 min and 4 hr respectively after training, and by 50% of the controls. This deleterious effect of narcosis on a recently acquired association is reminiscent of the retroactive inhibition on learning caused in mammals by electroshock (Duncan. J. comp. physiol. Psychol. 42:32, 1949; Gerard. Science 122:225, 1955). Both interference factors are liable to affect various processes in the brain; but their common disruptive effect on electrical activity could underly, at least partially, their similar action on memory.

89. CORTICO-SPINAL TRACT OF CAT. W. W. Chambers and C. N. Liu.* Dept. of Anatomy and Inst. of Neurological Sciences, Univ. of Pennsylvania, Philadelphia.

The site of origin and spinal distribution of the cortico-spinal tract has been determined by Nauta and Marchi techniques in 12 cats. The tract originates in the sigmoid, coronal and anterior ectosylvian gyri, and not from parietal, occipital and temporal areas. Two tracts are found following unilateral lesion (ablation of sigmoid gyrus or section of bulbar pyramid): a large crossed, lateral tract and a small uncrossed, ventral tract. The ventral tract extends into upper cervical segments and crosses in ventral white commissure before entering spinal gray. In contrast 4 tracts are seen following unilateral lesion of anterior ectosylvian gyrus (crossed and uncrossed, lateral and ventral). All 4 tracts are small, the crossed lateral and uncrossed ventral being the larger. The preterminal distribution of all cortico-spinal tracts (not revealed by Marchi) was chiefly to the dorsal horn with some fibers to the intermediate gray. In addition many fibers were distributed to gracile and cuneate nuclei and a few to Clarke's nucleus. No preterminal degeneration was found in the ventral horn. This distribution of the cortico-spinal tract suggests an influence of this system on "sensory" as well as "motor" mechanisms. Discrete lesions of the sigmoid gyrus, with deficits limited to a single limb, reveal that the cortico-spinal system is spatially organized not only at its cortical origin but also at its spinal termination. (Supported by USPHS.)

90. EFFECT OF PYROGEN ON EMBRYONIC DEGENERATION IN THE RABBIT. M. C. Chang. Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.

Piromen, Pseudomonas Polysaccharide (supplied by Drs. Fevold and Herwick of Baster Labs.) was administered intravenously to female rabbits 10-11 hr after fertile mating in order to determine the pronuclear behavior of ova at a higher body temperature. When the ova were examined 8 hr after ovulation no indications of polyspermy and fragmentation of the pronucleus were observed in a small number of ova. However, when 13 animals were examined at late pregnancy, 15-23 days after mating, 35% of normal fetuses and 40% of degenerations after implantation in terms of the number of corpora lutea were observed as against 74% of normal fetuses and 11% of degenerations in control animals. Since a statistically significant difference was displayed in these percentages it appears that this pyrogen has a definite latent effect on the embryonic development. For a period of 3-4 hr the rectal temperature is raised from a normal temperature of 103°-106.6°F following the administration of 400-1600 gamma of Piromen. Whether the effect is entirely due to the increase of body temperature has not been determined.

91. EVIDENCE FOR DYNAMIC PULMONARY STRETCH RECEPTORS. John L. Chapin (intr. by Clarence A. Maaske). Dept. of Physiology, Univ. of Colorado Med. Ctr., Denver.

Maintained inflation of the lungs of the anesthetized dog with air at 20 mm Hg pressure results in a period of apnea lasting up to a minute or more. The respiratory efforts at the breaking point are slow and deep as long as the pressure is maintained. If the pressure is released, the dog takes rapid deep breaths for several seconds. If, at the breaking point, the lung is allowed to deflate but is immediately reinflated to the same pressure with a gas of approximately breaking point composition, another apneic period results. This ability to breath-hold past the breaking point is probably not due to improved blood gases. Circulation time from lung to chemoreceptors is about 5 sec. Any apnea during this time cannot result from improved lung gas chemistry. After 5 sec there is little likelihood of chemical improvement since the inspired gas is poor chemically. Vagal impulses resulting from lung stretch have been described as being proportional to the amount of stretch and lasting as long as the stretch was maintained. It seems unlikely that this sort of stretch receptor could be responsible for the second apnea period described above. Before deflation, the ventilatory inhibition afforded by the stretch was not adequate to prevent respiratory efforts but the same stretch a second later was adequate. The only difference was lung movement immediately preceding the second apnea. Data from other sources could be interpreted as showing ventilatory inhibition resulting from lung movement and not lung stretch per se.

92. SINUSOIDAL ANALYSIS OF THE LOW FREQUENCY RESPONSE OF THE BLOOD VOLUME RECEPTOR. Kent M. Chapman (intr. by James P. Henry). Aero Med. Lab., Wright Air Development Ctr., Wright-Patterson AFB, Ohio.

A quantitative evaluation of 3 coefficients specifying the low frequency response characteristics of the left atrial blood volume receptor of Gauer and Henry, based on analysis of the transient responses of step-function volume increments, has recently been reported (Nat. Biophysical Conf., 1957, in press). In the present report the same coefficients are evaluated, using sinusoidal analysis as an alternative approach. Dial or chloralose anesthetized dogs with a surgically implanted rubber balloon in the left atrium were used. Low frequency sinusoidal variations of atrial volume were produced with an improved motor-driven syringe pump. Afferent activity in single nerve fibers from left atrial stretch receptors were recorded simultaneously with left atrial pressure during sinusoidal excitation. Amplitude and phase relations between stretch receptor discharge intensity, left atrial pressure and balloon volume were plotted as complex loci, with frequency as a parameter. Explicit evaluation of 3 dynamic coefficients was made from 3 equations arising from the frequency producing the maximum out-of-phase component, from the

real axis intercepts of the complex loci, and from the in-phase pressure-volume ratios. Calculations show qualitative agreement between the transient and sinusoidal analysis techniques but large quantitative discrepancies were frequently met.

93. CHANGES IN TISSUE VULNERABILITY INDUCED BY HYPNOTIC SUGGESTION. Loring F. Chapman,* Helen Goodell* and Harold G. Wolff. Cornell Univ. Med. College, New York City.

Peripheral nerve action can result in alterations that both damage the tissue subserved and make it more susceptible to injury. Significantly more skin damage following exposure to similar amounts of tissue-damaging stimulation occurs in the "flare zone" of an axone reflex produced by noxious stimulation than in control areas (Bilisoly, Goodell and Wolff. Arch. Int. Med. 94:759, 1954). Subsequent observations have demonstrated increased tissue vulnerability associated with alterations induced by hypnotic suggestion. Three subjects in deep hypnosis immersed 1 arm and then the other in a tank of water at a temperature of 34°C. For the test arm the water was suggested as being "very hot"; for the control arm, as "comfortable, neither hot nor cold." Suggestions were made that the test arm was "burned," "injured" and "painful"; the control arm was "intact" and "comfortable." Both arms were immediately exposed to similar amounts of focal tissue-damaging stimulation (thermal radiation or trichloracetic acid). The injured regions were examined and photographed at intervals thereafter. Significantly more skin damage was observed on the arms in which injury had been suggested than on the control arms. Pain threshold and finger pulse amplitude measurements indicated local minute-vessel dilatation during the phase of increased vulnerability. Such vasodilatation has been shown to be linked with increased vulnerability and probably plays a role in the vulnerability induced by hypnotic suggestion. From other related observations it seems likely that a chemical substance or substances also participates in the reaction.

94. ADRENAL REGENERATION HYPERTENSION: DRUG EFFECTS. J. J. Chart,* Gloria Ulsamer,* Louise Quinn,* Nancy Howie,* Betty Sullivan* and Robert Gaunt. Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

We have confirmed, in observations on more than 100 rats, the reports of Skelton (Proc. Soc. Exper. Biol. & Med. 90:342, 1955) that removal of one adrenal and enucleation of the other causes hypertension in animals sensitized by unilateral nephrectomy and a 1% NaCl drinking solution. No distinction could be made between the results in males or females or in animals weighing either 60 or 150 gm at the beginning of a standard 7-wk period of observation. Blood pressure ordinarily began to rise after 1-2 wk and approached 200 mm Hg by 7 wk. Mortality was 18%. Gross pathological changes (periarteritis nodosa, etc.), although sometimes present, were less severe than in DCA-hypertension. Hydrocortisone (1.0 mg/day) inhibited adrenal regeneration; this was associated with reductions in blood pressure. Smaller doses (0.1 mg/day) were ineffective. Amphenone B (100 mg/kg/day) increased weights of the regenerating adrenals and exerted other typical effects but did not affect blood pressure. Hydralazine (Apresoline), 10 mg/kg/day, reduced blood pressure but was not otherwise protective. Reserpine (Serpasil), 100 μg/kg/day, reduced blood pressure and the voluntary salt intake; in acute experiments both reserpine and hydralazine were hypotensive under conditions in which changes in salt intake were not involved. Reserpine and hydralazine combined were little, if any, more effective than either alone (in contrast to DCA-hypertension). None of the drugs maintained normotension in the doses used.

95. QUANTITATIVE EVALUATION OF COMPENSATORY RESPONSES OF DOGS TO HEMORRHAGE. Shu Chien (intr. by Magnus I. Gregersen). Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.

The immediate compensatory responses to hemorrhage consist mainly of vasoconstriction and hemodilution. The relative importance of these 2 mechanisms and their quantitative effectiveness were studied in unanesthetized splenectomized and splenectomized-sympathectomized dogs after single blood losses ranging from 5-50% of the measured blood volume. Bleeding rate was kept at 100 cc/min. The degree of hemodilution was calculated from the changes in hematocrit and plasma proteins. Thus the remaining blood volume, with hemodilution taken into consideration, was calculated and a true blood pressure, blood volume relationship established. By comparing this pressure-volume relationship of splenectomized dogs with that of splenectomized-sympathectomized dogs, one could evaluate the function of sympathetic reflex activity during various degrees of hemorrhage. It was found that splenectomized dogs could compensate for losses up to 15% of the original blood volume without significant changes in arterial pressure and heart rate. This was accomplished by both hemodilution and vasomotor activity, especially the latter. As the amount of hemorrhage was increased above 15%, the arterial pressure fell in a linear fashion with the amount of hemorrhage. With increasing blood losses, the degree of hemodilution increased, whereas the sympathetic activity became less effective. The heart rate did not increase until more than 20% of blood volume was removed.

96. PHYSIOLOGICAL EFFECTS OF OZONE. Hans G. Clamann and Richard W. Bancroft. Dept. of Physiology-Biophysics, School of Aviation Medicine, USAF, Randolph AFB, Texas.

There is much confusion in the literature on the actual toxicity of ozone. With respect to the ever-increasing importance of ozone for industrial purposes and with respect to its presence in the upper atmosphere as a possible hazard for aircraft, accurate data on the physiological effects of ozone are very desirable. Experiments were first carried out on various laboratory animals (by the Armour Research Foundation, Chicago). For a 3-hr exposure time, the LD_{50} was found to be 12.6 ppm by volume for mice and 25.7 ppm for guinea pigs. Death was caused by edema and hemorrhage of the lungs. Studies on 5 human subjects showed great differences in individual sensitivity. Irritation of the respiratory tract was observed at concentrations as low as 0.6 ppm after 30 min. Gross changes of respiratory function (reduction of vital capacity in more than 50%, pulmonary edema) occurred after 1 hr at 6 ppm. The soft tissues of the respiratory tract seem to be the only tissues attacked by ozone. The sense of smell is definitely affected. An effect on the conjunctiva of the eye was neither felt nor observed by inspection. No effects upon blood and circulatory system were found.

97. CHRONICALLY IMPLANTED POLAROGRAPH ELECTRODES. Leland C. Clark, Jr. and George

Misrahy.* Antioch College, Yellow Springs, Ohio, and Wright Air Development Ctr., Wright-Patterson AFB, Ohio.

Various types of wire were implanted in the brains of cats, using subcutaneous silver plates as a reference electrode. For several days following implantation the oxygen current slowly increases, and slow (8-10/min) waves develop and increase in amplitude. The amplitude is greatly decreased on inhalation of gases low in oxygen or high in carbon dioxide and in barbiturate anesthesia where, in addition, the waves become sinusoidal. In chloralose anesthesia the frequency increases. The oxygen available to the cathode increases 3-fold on breathing oxygen and on breathing 10% carbon dioxide in air. Mescaline analogs had small effects on the oxygen availability and type of curve. Dexedrine increases oxygen availability and frequency of the waves, and decreases the amplitude. Electroshock greatly increases oxygen availability and abolishes the waves which become sinusoidal on return. Audiogenic seizure in rats abruptly diminishes oxygen availability and abolishes the waves. Corticograms of the cat are unaffected by the functioning polarograph cathode except when the electrode is within a millimeter of the oxygen electrode. D.C. potential recordings showed none of the slow waves unless an electrochemical potential pre-existed between the two recording electrodes, thus suggesting that the wave phenomena represent rhythmic variation in brain oxygen availability. (Partially supported by Natl. Insts. of Health Grant M-600.)

98. ALVEOLAR SURFACE TENSION. John A. Clements, Elwyn S. Brown and Rudolph P. Johnson (intr. by D. B. Dill). Directorate of Med. Research, Chem. Warfare Labs., Army Chem. Ctr., Md.

The surface tension of lung extracts depends on the state of expansion or compression of the extract surface. Good correlation has been found between the tension-area behavior of extracts, and of intact lungs as calculated from pressure-volume data. This information together with chemical studies of the extract surface and the staining reactions of the lungs suggest that their peculiar surface properties are due to a surface-denatured layer of mucoprotein. By combining the tension-area data with the measurements of Macklin and Hartroft of alveolar sizes and distributions of sizes we have computed lung pressure-volume diagrams for rat, cat, dog and man. These show convincing similarity to experimental P-V diagrams. The relationship between lung volume and mean alveolar radius calculated on this basis agrees well with that directly determined by Radford and McLaughlin from microphotographs of subpleural airspaces. A nonionic detergent, 0.5% Tween 20, has been found to fix the surface tension of lung extracts at 30±2 dynes/cm and to eliminate virtually all surface hysteresis. This information, fitted into the calculation, has resulted in pressure-volume and volume-mean radius relationships that are in close agreement with the experimental data of Radford and McLaughlin on rat lungs. Several of the peculiarities of pulmonary mechanics can be explained on 2 experimentally supported assumptions: 1) the lung surface is covered with mucoprotein; 2) there is a logarithmico-normal distribution of alveolar size.

99. PANCREATIC ELASTASE. Herman Cohen, Herbert Megel* and William Kleinberg.* Princeton Labs., Inc., Princeton, N. J.

Lansing and coworkers (Proc. Soc. Exper. Biol. & Med. 84:689, 1953) have found that in the teleost fish elastase occurs in the islet tissue. A similar conclusion has been reached by Carter (Science 123: 669, 1954) for the elastase of canine pancreas. Lewis et al. (J. Biol. Chem. 222:705, 1956) however have noted the presence of elastolytic activity in dog pancreatic juice. We have attempted to measure the elastolytic action of extracts of rat and guinea pig pancreas and to correlate this with the lipase activity. The animals were subjected to starvation, hyopophysectomy, cobalt treatment and pilocarpine injections. Starvation results in an increase of elastase activity and lipase action. Hypophysectomy causes a decrease in both elastolytic and lipolytic activity. Pilocarpine causes a depletion of elastase and lipase from the pancreatic tissue with appearance of lipolytic and elastolytic activity in the urine. Elastase is demonstrable in the duodenal juice of rats both before and after pilocarpine treatment. There is a decreased lipolytic and elastolytic activity of the pancreas of cobalt-treated guinea pigs but not rats. The parallelism between lipolytic and elastolytic activity under the above experimental conditions suggests that at least in the rat and guinea pig, there may be a similar cellular source for both elastase and lipase, namely the acinar tissue.

100. INTRINSIC PERIODICITY OF THE ISOLATED PONTILE RESPIRATORY MECHANISM IN THE CAT. Morton I. Cohen* and S. C. Wang. College of Physicians and Surgeons, Columbia Univ., New York City.

On the basis of ablation and stimulation experiments, the pneumotaxic center has been localized to a circumscribed region: the dorsolateral portion of the rostral pons, 1-2 mm caudal of the inferior colliculi. Neurons firing spontaneously and synchronously with phases of the respiratory cycle have been recorded in this region (Cohen and Wang, 1956). To determine if the pontile respiratory mechanism is intrinsically rhythmic, the effect on this respiratory neuronal activity of isolation of the pneumotaxic region was investigated in vagotomized cats. a) In the anesthetized cat, after hemisection at the level of the acoustic tubercles, neurons firing synchronously with respiratory movements could be recorded. b) In the midcollicular decerebrate cat after hemisection at the same level, neurons firing regularly and periodically were found. The time between successive bursts of potentials was of the order of magnitude of the respiratory period (5-10 sec); but the periodicity was not that of the respiratory cycle. c) In the decerebrate cat with additional mid-sagittal section, spontaneous neuronal activity in the isolated pneumotaxic region remained detectable, but the periodicity of these neurons was more irregular and different from that of the respiratory cycle. It is not clear as to whether this spontaneous activity is due to respiratory neurons.

101. EFFECT OF BONE MARROW-SPLEEN INJECTION IN MICE AFTER FAST NEUTRON IRRADIATION. Leonard J. Cole and Marie E. Ellis (intr. by G. A. Feigen). U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Adult LAf_1 mice were exposed (dose rate 80 rep/min) in the 60-inch Berkeley cyclotron to fast neutrons (2 Mev) produced by the Be (p,n) B reaction. The mice received a single postirradiation intravenous injection of fresh isologous bone marrow containing 10^6 nucleated cells, or a single i.p. injection of isologous infant spleen homogenate - 45 mg/mouse. Controls received suffer injection only. Three neutron dose levels were employed: 1) 510 rep which produced 100% early mortality (death by 8 days);

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2) 475-500 rep which in the controls elicited some survival (24%) at 8 days, but none by 30 days; 3) 425 rep, after which 11 out of 47 controls (23%) died by 8 days; 10 more succumbed by 30 days with a mean survival time (MST) of 19 days. Marrow injection had no protective effect after 510 rep: all the treated mice died by 8 days. After 475-500 rep, marrow injection elicited slightly higher survival than in controls: 45% at 8 days, 11% at 30 days. Following 425 rep, marrow treatment increased 30-day survival (31 out of 48 or 65%) only slightly compared to controls, with no change in MST of non-survivors dying after 8 days; however, fewer deaths before 8 days (6 out of 48) occurred in the marrow-injected mice. These and similar data obtained also on mice exposed to 8 Mev neutrons, and injected with splenic homogenate are in marked contrast to those observed with lethally x-irradiated mice. The fact that neutron deaths during the third week occur apparently in spite of histologically-evident bone marrow regeneration, implies a mode of death different from x-rays.

102. PREVENTION OF EXPERIMENTAL ENCEPHALOMYELITIS BY PRIOR INJECTIONS OF HOMOLOGOUS SPINAL CORD. Richard M. Condie,* John T. Kelly,* Berry Campbell and Robert A. Good.* University of Minnesota, Minneapolis.

Experimental disseminated encephalomyelitis is produced in experimental animals by injection of spinal cord with Freund's adjuvants. Although allergic mechanisms have been implicated in the pathogenesis of this disease, its basis remains obscure. Consideration of the possibility that disseminated encephalopathy might derive from mechanisms involving hypersensitivity rather than production of circulating antibody, it was postulated that prior immunization with aqueous emulsion of spinal cord might afford protection against the disease. To test this hypothesis, the following experiments were done. Sixteen rabbits (group I) were given repeated s.c. injections of homologous spinal cord. A series of injections covered 4 months, being terminated 45 days prior to administration of spinal cord + adjuvant. Fifteen rabbits (group II) received 2.5 gm of spinal cord at the time of injection of spinal cord + adjuvant and 15 rabbits (group III) received spinal cord and adjuvant alone. In group I, 12% became paralyzed and none died; in group II, 93% became paralyzed and 80% died; in group III 80% became paralyzed and 53% died. Daily subcutaneous injections of spinal cord administered for 20 days following injection of spinal cord + adjuvant did not prevent death or paralysis. The results of these experiments are interpreted as evidence that either immunization or Felton's "immunological paralysis" to spinal cord antigen results in prevention of experimental allergic encephalitis. (Aided by grants from the PHS, Helen Hay Whitney Fndn., Minnesota Heart Assoc., American Heart Assoc., and Minnesota Div. of American Cancer Soc.)

103. PRESENCE OF TROPHIC HORMONES IN FETAL RAT PITUITARY. Alex N. Contopoulos* and Mirian E. Simpson. Inst. of Exptl. Biology and Dept. of Anatomy, Univ. of California, Berkeley.

It has been reported (Fed. Proc. 15:39, 1956) that the growth promoting activity of plasma from pregnant rats is increased 3 times above normal. In order to determine whether the growth-promoting activity originated at least in part from the pituitary of the fetus, fetal pituitaries were assayed for trophic hormones on days 19, 20, 21 of gestation, and on day of birth. The pituitaries were weighed, frozen immediately and stored in dry ice. On the day of injection the pituitaries were ground in saline and aliquots representing total doses of 1, 2, 4 or 6 pituitaries were injected into recipient hypophysectomized rats. The injections were begun 13-14 days post-hypophysectomy and were distributed in daily doses, intraperitoneally, over 4 days. Each rat received 0.5 μc of I^{131} 16-18 hr before autopsy. At autopsy, 96 hr after onset, tibia, thyroids, adrenals and ovaries were fixed. The growth hormone content was evaluated by the tibia test. Thyrotrophic hormone was measured by the increased I^{131} uptake in the thyroids of the recipients and by histological evidence of stimulation. Both growth and thyrotrophic hormones were present in the fetal pituitary by the 19th day and the content increased until on the day of birth the growth hormone content had increased 6-fold, and thyrotrophic 2- to 3-fold. Other tropic hormones, FSH, ICSH and ACTH, were not detectable at the dose levels injected. (Aided by PHS Grant A-661(C) and RG-4965.)

104. PULMONARY MECHANICS DURING INDUCED PULMONARY EDEMA IN ANESTHETIZED DOGS. C. D. Cook,* G. Schreiner* and J. Mead. Dept. of Physiology, Harvard School of Public Health, Boston, Mass.

Pulmonary compliance and resistance were measured in spontaneously breathing anesthetized dogs in the prone position, along with the total intrathoracic gas volume at end-expiration (method of Dubois and Marshall) and the ventilated volume of the lungs at end-expiration (helium closed-circuit method). Elevation of left atrial pressure to levels between 35 and 50 cm H_2O was produced by intravenous infusion of 3% dextran in Ringer-Tyrode solution (approximately 30 ml/kg body weight) at a rate of 50-100 ml/min together with partial obstruction of the ascending aorta by means of an intra-aortic balloon. The degree of pulmonary edema in 6 dogs as judged by lung-body weight ratios was moderate to severe (mean 2.6%). Pulmonary compliance fell to approximately 30% of control levels and pulmonary resistance increased approximately 3-fold. The average lung volumes determined by the 2 methods were the same prior to the production of pulmonary edema, but in the presence of edema the ventilated volume decreased more than the total intrathoracic gas volume (32 versus 20%). This discrepancy ranged from 4-30% and probably reflects the trapping of gas by froth within the lungs.

105. IONIC TRANSFER ACROSS THE ISOLATED LARGE BULLFROG LARGE INTESTINE. I. L. Cooperstein,* David Chalfin* and C. Adrian M. Hogben. Natl. Heart Inst., Bethesda, Md.

Because the large intestine of some species is able to develop a pH gradient, the movement of Na^{22} and Cl^{36} has been simultaneously measured across the isolated colon of the bullfrog. When exposed as a flat sheet to bicarbonate-Ringer's solution, the colon is characterized by a spontaneous potential of 45 mv, serosa positive to mucosa, and a short-circuit current of 100 μ amp cm^{-2}. In contrast to the finding (in the case of the toad and guinea-pig) of a net movement of sodium equal to or greater than the short-circuit current (Ussing and Andersen, Int. Congress Biochem., 1955), the unidirectional flux of sodium from mucosa to serosa is less than or only equal to the short-circuit current even through it is 6 times the opposing serosa to mucosa flux. The chloride flux is one third the electrical current and there is not a net movement which would account for the current deficit at 0 mv. At 45 mv, serosa positive, the net sodium transport against its electrochemical

potential gradient is twice as large as the net chloride movement along its electrochemical gradient. The chloride flux ratio at 45 mv is less than 2, i.e. considerably less than that expected for free passive diffusion alone. Tied sacs of the same large intestines, whether everted or normally disposed, deplete both chloride and bicarbonate at the mucosal surface and accumulate both anions at the serosal surface. This transfer of bicarbonate, at the spontaneous potential, is of the same order of magnitude as the calculated current deficit at 45 mv.

106. IN VITRO STUDIES ON ISOLATED RENAL TUBULE (FLOUNDER) AND ON THIN SLICES OF RENAL CORTEX (RABBIT). John H. Copenhaver,* S. K. Hong* and R. P. Forster. Dept. of Zoology, Dartmouth College, Hanover, N. H., Dept. of Physiology, Univ. of Buffalo School of Medicine, Buffalo, N. Y., and Mount Desert Island Biological Lab., Salisbury Cove, Me.

Within a series ofphenolsulfonphthaleins,members excreted slowly (e.g. bromcresol green and bromphenol blue) inhibit transport competitively in direct relationship to their tendency to accumulate within proximal cells of the isolated tubule preparation. Actively excreted compounds, which are concentrated only intraluminally, run out into the ambient medium quickly within 2,4-dinitrophenol or cold, whereas an intracellular "trapping mechanism" retards the runout of sluggishly transported compounds. Transfer from cell to lumen possesses the energy-demanding characteristics shown previously to pertain to penetration at the peritubular cell border, and it likewise is subject to competitive inhibition. With the mammalian slice preparation, which exhibits only intracellular accumulation as an active process in dye transport, those compounds most slowly excreted in vivo and in the isolated tubule preparation are most intensively accumulated by the tubular cells. These also are the most effective in preventing p-aminohippurate accumulation and in facilitating run-out of PAH from slices. However, none of these competitors facilitate runout of PAH at 0°C. Benemid, 2,4-dinitrophenol, picrate, α-ketoglutarate and succinate, which facilitate run-out at 25°, are also without effect in the cold. Accumulation of bromcresol green in slices at 0° is not diminished significantly by high concentrations of PAH.

107. FATE OF SEROTONIN PERFUSED THROUGH AN ISOLATED BRAIN WITH INTACT NERVOUS CONNECTIONS. E. Costa* and M. H. Aprison. Galesburg State Research Hosp., Galesburg, Ill.

Cross-circulation experiments modified from the methods of Heymans and Heymans and of Swiss and Maison were performed on rabbits to study the distribution of serotonin injected into the blood perfusing the recipient's head. Blood pressure and EEG of both animals were recorded. Changes in the recipient's EEG were not observed during the first 30 min of cross-circulation unless enduring drastic blood pressure changes occurred. Our experiments never exceeded this period. Recovery of injected serotonin from the recipient's venous blood was approximately 3%; when injected together with Marsilid, the recovery was about 12%. The brain of the donors and recipients was divided into 4 parts: Telencephalon (T), Mid-brain (M.B.), Medulla-Pons (P), Cerebellum (CB),.and analyzed for serotonin content by Garven's modification of Gaddum's technique. Results (μg/gm) for 20 donors are: T = 0.36±.12, MB = 0.76±.18, P = 0.54±.18 and CB = 0.14±.07. Infusion of Marsilid (80 mg) to the recipient (N = 4), resulted in a slight increase in T. When serotonin alone was used (2 mg; N = 3), the neurohormone content in T also increased slightly. When serotonin (4 mg) plus Marsilid (80 mg) were injected, the neurohormone levels (μg/gm) in all 4 experiments rose significantly to T = 1.97±.56, M.B. = 2.84±1.37, P = .8±.11 and CB = 0.85±.38. Perfusion of the isolated head with serotonin caused a fall in blood pressure in the recipient.

108. HYPOTHALAMIC RESPONSES DURING AND FOLLOWING ANEMIC DECEREBRATION IN THE CAT. LeRoy L. Costantin and S. C. Wang (intr. by W. L. Nastuk). College of Physicians and Surgeons, Columbia Univ., New York City.

In 15 cats, the hypothalamus was stimulated with bipolar coaxial needle electrodes and a Grass rectangular wave stimulator following "decerebration" by the anemic method of Pollock and Davis (1923). Control responses were obtained by stimulating the hypothalamus with the carotid arteries open. In some cases, the hypothalamic pressor responses disappeared within 30 min of occlusion, and slow recovery was observed after removal of carotid occlusion. In others, responses persisted for about 2 hr during occlusion. The only consistent response noted was extensor rigidity. Pupil size varied. On autopsy, a rich anastomotic network between vertebral and carotid branches in the occipital region was demonstrated. Thus, the classical anemic decerebration cannot be depended upon to produce ischemia in structures rostral to the mid-pontine level. In 11 cats, additional ligatures were placed around all structures in the neck except the carotid arteries and vagus nerves. After controls were obtained, the carotid arteries were occluded for a variable period of time. This gave, invariably, maximal pupillary dilatation and disappearance of the pupillary reflex, a marked blood pressure rise, and a marked extensor rigidity. Hypothalamic responses disappeared within 10 min. In 5 cats the hypothalamus did not recover after 1 hr of occlusion, while 2 recovered after 30 min of occlusion, 2 after 45 min, and 2 after 60 min. In these animals a small blood supply to the hypothalamic region remained intact.

109. STANDARDISATION OF TEST EXERCISE. J. E. Cotes, F. Meade and M. E. Wise (intr. by R. H. Shepard). British Med. Research Council, Pneumoconiosis Research Unit, Cardiff, Wales.

To facilitate comparison with other forms of test exercise we sought a connection between the energy expenditure of horizontal treadmill walking (Eo_2) and the vertical work done per minute by raising and lowering the body with each step (W_L), (cf Benedict and Murschhauser. H. Carnegie Publ. 231, 1915; Smith. ibid, 309, 1922). W is the product of lift/step (L), body weight (M), and step frequency/min (n) and it can be shown that $L = (P - d)^2/8R$, where P, d and R are length of pace, foot and leg respectively. Pace length is velocity (V) divided by step frequency. Then: $W_L = M.n. (P - d)^2/8R \times 427 = M. (V - n.d)^2/n.8.R \times 427$ Cal/min. We found for 10 subjects walking with natural step frequency at 1 - 4 mph a linear relationship $Eo_2 = 3.25\ W_L + 2.4$ (95% confidence limits = 0.7 Cal/min), although there are significant differences between subjects. Uphill, work is done against the gradient (W_M) as well as with each step and the energy relationship becomes $E'o_2 = k_1 W_M + k_2 W_M + C$. On 3 subjects the range of values for k_1, k_2 and C were respectively 2.70 - 3.09, 2.60 - 2.68 and 1.78 - 3.08. The findings explain the significant negative correlation between leg length and energy expenditure (Brockett. et al. Med. Nutrition Lab.

Report 177:1956), but not the effect on energy expenditure of deviations from natural step frequency.

110. FILTRATION COEFFICIENT OF CAPILLARIES OF THE CENTRAL NERVOUS SYSTEM. N. A. Coulter, Jr. (intr. by R. W. Stacy). Div. of Biophysics, Dept. of Physiology, Ohio State Univ., Columbus.

The circulation of the CNS flows through a relatively rigid container, the cranium and vertebral column. This permits measurement of the filtration coefficient of CNS capillaries. A trephine opening is made in the skull, and connection is made to a volumeter. This in turn is connected to a pressure reservoir and manometer. Pressure is applied and the rate of change of volume of intracranial contents is observed. Since the skull, etc. is rigid (Monro-Kellie doctrine), this volume change must reflect a transport of fluid from extravascular compartments to the circulation, assuming blood flow remains approximately constant during the period of observation. Knowing the brain weight, the volume of the minute vessels (Gibson, Medical Physics, II:443, 1950), and the capillary radius (assumed 5 micra), the capillary surface area can be estimated. From these data the filtration coefficient of CNS capillaries is determined. For cats this is 10.6×10^{-8} ml/sec/cm H_2O/sq cm. This compares with 2.5×10^{-8} for muscle capillaries (Pappenheimer et al. Am. J. Physiol. 167:13, 1951) and 56×10^{-8} for frog mesentery capillaries (Landis. Am. J. Physiol. 82:217, 1927). The relation of filtration rate to applied pressure is linear for positive pressures. (Aided by Natl. Insts. of Health Grant G-3998.)

111. CARDIOVASCULAR RESPONSE OF COLD ACCLIMATIZED DOGS TO IMMERSION HYPOTHERMIA. Benjamin G. Covino and William R. Beavers (intr. by Kaare Rodahl). Arctic Aeromed. Lab., APO 731, Seattle, Wash.

Many factors, such as age, pH, electrolytes, have been shown to influence the terminal cardiac event of dogs subjected to immersion hypothermia. The feeling is prevalent among many workers in this field that the incidence of ventricular fibrillation is diminished in dogs who have been exposed to a cold environment prior to being rendered hypothermic. However, no experimental evidence is available to confirm or deny this hypothesis. During this present study adult mongrel dogs were anesthetized with pentobarbital and cooled to terminus in a cold water bath of 5°C. The control series included 25 dogs who were exposed to an environment of 15.5° - 29.4°C. Twenty-four dogs (96%) in this group succumbed to ventricular fibrillation at an average rectal temperature of 18.0°C. A second group of 6 animals were exposed to an environmental temperature of -45° - 0°C for a period of 4 wk and then rendered hypothermic. All 6 dogs terminated in asystole at an average rectal temperature of 14.6°C without exhibiting any cardiac arrhythmias. Five additional dogs were exposed to an environmental temperature of -45 - 20°C for 1 wk and then cooled. Four of the 5 dogs (80%) fibrillated at a rectal temperature of 15.0°C. The results indicate that dogs exposed for prolonged periods to a low environmental temperature are more resistant to hypothermic ventricular fibrillation. Brief exposure periods do not decrease the incidence of fibrillation but do lower the temperature at which this cardiac abnormality occurs.

112. EPINEPHRINE-INDUCED GLYCOGENOLYSIS AND POTASSIUM LOSS IN THE ISOLATED PERFUSED LIVER. Albert B. Craig, Jr. (intr. by John R. Murlin). Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The isolated frog liver was perfused with "frog-Ringers" solution containing washed beef red cells and varying concentrations of potassium. Potassium and glucose concentrations were determined in each 10-min sample of effluent and in the affluent. In control experiments the liver showed a loss of potassium during the first 10 min with fluids containing less than 4.4 mEq K/l. The liver was able to gain potassium for the next 30 - 50 min after which time all preparations lost potassium at a slow rate. Livers were stimulated with solutions containing epinephrine for 10 min and then perfused again with control solutions. Potassium immediately left the liver and reached a maximum rate of loss during the 10-min period following stimulation. The maximum rate of glucose loss occurred 10 min later at which time the rate of potassium loss was decreasing. More potassium and glucose were lost when the external potassium concentration was high (3.5 - 5.2 mEq K/l.) than when it was low (.65 - 2.0 mEq K/l.) When the rate of glucose loss began to decrease after stimulation, potassium returned to the liver. When epinephrine stimulation was prolonged, potassium loss followed the same pattern, except that there was no gain of potassium until the rate of glucose loss decreased. In controls, potassium loss was accompanied by sodium gain, but with epinephrine, sodium as well as potassium was lost. These results were interpreted as demonstrating that potassium loss is probably closely connected to processes of intermediary carbohydrate metabolism which are stimulated during epinephrine-induced glycogenolysis.

113. PATHOGENESIS OF EXPERIMENTAL HYPERTENSION PRODUCED BY CAROTID SINUS AREA CONSTRICTION IN DOGS. E. E. Crandall,* H. L. McCrorey,* E. J. Sukowski* and G. E. Wakerlin. Chicago, Ill.

Bilateral constriction of the carotid sinus area, including the internal carotid and external carotid (and occipital) arteries, in the dog resulted in hypertensions of 30 - 100 mm Hg in 8 of 10 dogs (Circulation Res. 2:416, 1954). Presently these hypertensions have durations of 34-48 months and have been confirmed in 3 additional dogs. General health, appetite, body weight, body temperature, urinalyses and heart rate have continued normal. Bilateral constriction of the internal and external carotid (and occipital) arteries above the sinus in 3 dogs produced hypertension, as did bilateral constriction of the denervated sinus in 1 dog. Bilateral constriction of the vertebral arteries in 6 dogs resulted in normotension in 5 and hypertension of 40 mm Hg in 1. Five dogs subjected to bilateral removal of the carotid sinus developed modest hypertensions averaging 30 mm Hg. Cerebral angiography suggested that the hypertension is compatible with a grossly normal cerebral arterial tree. Results with sodium cyanide suggested absent carotid body function. Phenoxybenzamine produced an acute reduction in pressure to normotension. Chronic treatment with phenoxybenzamine for 6 months showed significant reduction in pressure. Intraperitoneal pentobarbital anesthesia produced no significant average change in blood pressure. Passive transfer of homologous anti-renin to hog renin was without antihypertensive effect. These findings suggest that pathogenesis involves an alteration in cerebral hemodynamics (decreased cerebrovascular pulse volume?) with increased vasoconstrictor tonus and a possible humoral pressor factor.

114. COMPARISON OF MONOPHASIC ACTION POTENTIALS RECORDED BY INTRACELLULAR AND SUCTION ELECTRODES. P. F. Cranefield, B. F. Hoffman, E. Lepeschkin, B. Surawicz* and H. Herrlich.* Dept. of Physiology, State Univ. of New York, Downstate Med. Ctr., New York City, and Div. of Exptl. Medicine, Univ. of Vermont, Burlington.

In isolated perfused rabbit hearts, ventricular monophasic action potentials were recorded simultaneously by a flexible suction electrode and a flexibly mounted intracellular microelectrode. The amplitude was 80-120 mv in the intracellular, and 20-40 mv in the suction curve. A hyperpolarization of about 1 mv preceded the depolarization if the diphasic curve, registered before penetration of the electrode into the cell or application of suction, had shown a Q wave. The depolarization terminated in a peaked reversal of 1-5 mv, which coincided the downstroke of R in the diphasic curve. In ventricular extrasystoles the negative deflection often disappeared; it might therefore be attributed to activation of distant portions of the ventricle. Repolarization followed the same time course in the suction and intracellular curves under all conditions except that the descent of the suction curve was always followed by a negative afterpotential comprising 5-17% of its height. This potential was not present in the intracellular curve unless the electrode became loose; it coincided with the descending branch of the ventricular myogram and is attributed to transient membrane depolarization caused by movement of the electrode. Within 5-20 min after application of suction the amplitude decreased about one half and the plateau became less steep. It is concluded that at least during the first 5 min after application of suction, the configurations of the monophasic potential and of the transmembrane potential are identical with the exception of their initial and terminal portions near the base line.

115. INTERACTION BETWEEN ADRENALINE AND RETICULAR FORMATION AS A SOURCE OF BULBAR INHIBITION. J. I. Cranmer and L. M. N. Bach (intr. by J. K. Hampton, Jr.). Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.

Earlier work indicated that the action of adrenaline on the reticular formation would reverse reflex facilitation to reflex inhibition (Kelly and Bach. Am. J. Physiol. 183:633, 1956). The present investigation has shown repeatedly that intravenous infusion of dibenzyline in cats anesthetized with Numbutal would abolish inhibition of the patellar reflex elicited by electrical stimulation of the caudal bulbar reticular formation through stereotaxically oriented electrodes. The inhibition could be easily re-established by subsequent intravenous infusion of adrenaline. Elimination and restoration of vasoconstriction simultaneously elicited and recorded exactly followed the changes in reflex inhibition. Using hypodermic stimulating electrodes, it was observed that certain medullary points producing inhibition of the patellar reflex were sensitive to the injection of minute amounts of adrenaline and a lowering of the threshold for inhibition was produced. No change in threshold was observed when similar amounts of saline were injected at these points. Other medullary areas which were productive of patellar inhibition were not affected by adrenaline injection but these points also failed to produce vasoconstrictor effects when stimulated. (Supported by Grant No. B-603-c from the Nat. Inst. of Neurological Diseases and Blindness, PHS.)

116. WHOLE-BODY RADIATION PROTECTION IN PRIMATES. B. G. Crouch* and Richard R. Overman. Clin. Physiology Labs., Univ. of Tennessee College of Medicine, Memphis.

The ability of certain sulfhydryl compounds to protect mice from radiation death, when administered prior to a lethal dose of whole-body x-irradiation, is well documented. Treatment of lethally irradiated mice with isologous or homologous bone marrow also provides a remarkable degree of protection from radiation death. It seems mandatory, therefore, that similar studies should be carried out in primates. Macaca mulata monkeys given 200-250 µg/kg body wt. of S2, β-aminoethylisothiuronium · Br · HBr prior to administration of 650 r (LD_{100}) whole-body x-irradiation are surviving at 173 days and are apparently normal. In each case the peripheral blood picture shows a return to normal by the 50th day post-irradiation. Additional macaques have been injected intravenously with a suspension of homologous bone marrow cells following administration of the LD_{100} dose of whole-body x-irradiation. The bone marrow, removed from the long bones of very young animals, was suspended in 50-70 cc of the donor's whole flood. By the 7th-10th day following radiation the peripheral blood of animals treated with bone marrow shows a decline in the total number of cellular elements, comparable to untreated radiated controls. By the 20th day in each case cellular repopulation of the peripheral blood has begun, with a dramatic increase in the number of circulating reticulocytes and immature white blood cells. Survivals up to 102 days post-irradiation have been obtained. (Supported by U. S. Atomic Energy Comm.)

117. DIRECT ACTION OF TRIIODOTHYRONINE ON PITUITARY TSH SECRETION IN HYPOTHALAMIC LESIONED RATS. Savino A. D'Angelo and Ronald E. Traum.* Jefferson Med. College, Philadelphia, Pa.

Lesions in the anterior hypothalamus of the rat result in diminution of blood TSH levels and thyroidal I^{131} release rate without materially affecting adenohypophysial TSH concentration (D'Angelo and Traum, Endocrinology 59:593, 1956). The present study sought to determine whether thyroid hormone can regulate TSH secretion despite hypothalamic deficit. Bilateral electrolytic lesions were produced in adult female rats by stereotaxy. One week post-operatively, lesioned rats and unoperated controls were injected with I^{131} (25 µc) preparatory to measurement of thyroidal radioiodine release rate by an in vivo decay technic. A decrease in radioiodine release rate was used to screen a total of 40 lesioned rats with disturbed pituitary-thyroid function. Two groups of screened rats were given daily subcutaneous injections of 2 or 20 µg of l-triiodothyronine for 14-16 days; a third group received saline. Unoperated controls received similar injections. At autopsy (25 days postoperatively), target endocrines were weighed and prepared for histologic study. Acid-saline extracts of pooled adenohypophyses were made for each group and bioassayed for TSH (stasis tadpole). The main results follow: 1) pituitary TSH concentration in saline-treated lesioned rats remained within normal limits, 2) triiodothyronine administration at both dose levels reduced pituitary TSH concentrations of lesioned rats to less than 5% of normal. The results strongly suggest that the hypothalamus has some measure of influence over the pituitary-thyroid system but that in the feed-back mechanism thyroid hormone acts largely at the pituitary level. (Supported by funds from grant A-432, Natl. Insts. of Health, PHS.)

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118. EFFECT OF DESOXYCORTICOSTERONE TRIMETHYLACETATE (DCT) ON LIVER SUCCINOXIDASE ACTIVITY IN RATS FED 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE. John P. DaVanzo* and W. J. Eversole. Dept. of Biology, Univ. of New Mexico, Albuquerque.

Six groups of rats were placed on a purified diet containing 0.58% 3'-methyl-4-dimethylaminoazobenzene and upon sacrificing liver succinoxidase determinations were performed. Group I, the control animals, was given no hormone. The succinoxidase activity, expressed as microliters of oxygen taken up/mg dry tissue/hr, decreased progressively from 65 at 1 month to 54 at 5 months. Group II consisted of intact animals given a single injection of 50 mg DCT. The enzyme values at 1 and 5 months were 79 and 58 respectively. The animals in Group III were adrenalectomized, given 50 mg DCT initially and 10 mg/month subsequently; succinoxidase activity was 70 at 1 month and 63 at 5 months. Seven adrenalectomized rats, Group IV, were each given one 150 mg injection of DCT. Upon autopsy at 5 months, 1 animal was found to have adrenal regeneration with concomitant hepatoma and a succinoxidase activity of 46. The remaining animals had no macroscopic indication of tumor formation and an average succinoxidase value of 69. Eleven animals in Group V were adrenalectomized and given 250 mg DCT over a period of 4-1/2 months. Four animals showed adrenal regeneration with accompanying tumors and 7 had no adrenal regeneration and no tumors. Succinoxidase values here were 60 and 75 respectively. Ten intact animals, Group VI, were each given 250 mg DCT. Here there was varying degree of tumor formation with an average succinoxidase activity of 58. Adrenalectomy with high doses of DCT appears to have a protective influence in azo-dye carcinogenesis.

119. EFFECT OF PARATHYROID EXTRACT ON RENAL EXCRETION OF PHOSPHATE IN THE CHICKEN. Douglas G. Davidson and Norman Levinsky (intr. by Robert W. Berliner). Lab. of Kidney and Electrolyte Metabolism, Natl. Heart Inst., Bethesda, Md.

Attempts to demonstrate a direct action of parathyroid hormone on renal tubular transport of phosphate have led to conflicting results. In the mammal, it is difficult to dissociate a possible tubular action of parathyroid extract from disturbances of glomerular filtration rate (GFR). Because the tubules of the kidney of the chicken are partly supplied via the renal portal circulation, separation of glomerular and tubular effects is facilitated. Renal phosphate excretion was studied by intravenous infusion of 2.5 and 25 μ mol/min of sodium phosphate. The infusions contained inulin to measure GFR. Renal tubular secretion of phosphate was demonstrated by bilateral phosphate excretion 40-60% in excess of filtered load. No difference was noted when infusions were given systemically or in a leg vein (renal portal system). In other experiments parathyroid extract, 0.5-2.5 U/hr/kg, was infused continuously into one leg vein. No constant or large changes in plasma phosphate concentration or in the GFR of either kidney occurred; 2.5 U/hr/kg uniformly resulted in greater phosphate excretion on the infused side than by the control kidney. Unilateral phosphate secretion was demonstrated, with excretion of 20-100% more phosphate than the filtered load. These experiments show that the renal tubule of the chicken can secrete phosphate, and that tubular transport of phosphate is acted upon directly by parathyroid extract.

120. MEMBRANE POTENTIAL OF SKELETAL MUSCLE FIBERS WITH LARGE-SIZE SUCROSE INSULATED MICROPIPETTES. Philip W. Davies. Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md.

In preparation for studying membrane potential of internally perfused muscle fibers the possibility of inserting large micropipettes without short circuiting the fibers has been explored. Glass micropipettes either 9-1/2 or 11 μ OD were sharpened in the form of hypodermic needles to 1/2 μ points. After filling with 118 mM KCl they were inserted longitudinally from 100-500 μ into single fibers on the inner surface of dissected frog sartorius muscles in Ringer's. Following Stämpfli's method, which employs sucrose solution as an insulating medium, an isotonic solution of sucrose (ordinary distilled water sufficed) was directed against the locus of insertion at a rate of 3.75 mm^3/min from a 200 μ orifice. Any short-circuiting of the fiber at this location was apparently prevented. The potential change recorded on penetration was corrected for Planck liquid junction potential (ca. 10 mv). The pipettes were free from "tip potential" observed with ultramicropipettes. On insertion the potential rose in one to several minutes to a peak level maintained for some time, followed by a decline. For 11 penetrations in 4 muscles remaining at virtually peak level for 30 min or longer, the average corrected value of membrane potential was 91.9 mv$\pm$1.6 mv S.E. (N =11), range 87.2 - 97.8 mv, with one exception, 81.3 mv. Individual values were taken as the average over a 15-min period including the peak. In one penetration the potential remained between 90 and 100 mv for 3 hr.

121. SMALL VEIN RESPONSES TO SYMPATHETIC NERVE STIMULATION. Darrell Davis (intr. by Thomas Findley). Depts. of Physiology and Pharmacology, Med. College of Georgia, Augusta.

Recent work (Am. J. Physiol. 185:453) indicates a large pressure increase in veins smaller than 0.5 mm diameter on sympathetic stimulation. To measure pressures in small veins with natural circulation cannulae 0.1 mm OD were inserted into rabbit ear veins 0.2-0.5 mm in diameter. Optical records were made of small vein pressure when centrally draining veins were or were not occluded by rubber clad clamps. When veins were demonstrably patent to perfusion from the cannulae tip the stimulation (Grass square wave stimulator 25 cps, 5 volts) of sympathetic fibers caused uniformly a fall in small vein pressure from 10-15 mm Hg (normal) to 5 mm Hg, followed by a rise to near the prestimulation figure. Partial occlusion of the venous drainage resulted in an increased resting small vein pressure, with a primary fall from sympathetic stimulation followed by a rise above the prestimulation level. More complete venous occlusion resulted in a higher resting small vein pressure, with a primary rise from sympathetic stimulation. Similar results were obtained when the pressure was measured by a retrograde catheter 0.5 mm OD. The resting level of pressure in a small vein, the response to, and the recovery from sympathetic stimulation depend upon the degree to which the venous drainage is occluded. The more nearly natural the circulation the more likely that sympathetic stimulation will lower small vein pressure. (Program supported by grants from the Natl. Heart Inst. and the American Heart Assoc.)

122. FUNCTIONAL CHANGES DURING CARDIAC FAILURE PRODUCED BY HEMORRHAGE IN DOGS

WITH PULMONIC STENOSIS. James O. Davis, M. Jay Goodkind* and Wilmot C. Ball, Jr.* Natl. Heart Inst., Bethesda, Md.

The present study was undertaken to determine the relationship of changes in cardiovascular and renal hemodynamic function to Na excretion following hemorrhage in dogs with pulmonary artery (PA) constriction and in normal dogs. Seven dogs with constriction of the pulmonary artery and 6 normal dogs were bled 15-25 cc/kg daily until a severe anemia (2.4-6.1 gm% hemoglobin) developed. Five of the 7 dogs with PA constriction developed signs of right heart failure, but no evidence of cardiac decompensation was observed in the normal dogs made severely anemic. Mean right atrial pressure (RAP) increased from 90-140 to 140-240 mm water in dogs with PA constriction, whereas RAP remained unaltered in the anemic but otherwise normal animals. Sodium retention occurred only in dogs with PA constriction, and ascites formation was associated with an RAP of 140 mm water or above (normal RAP was 10-70 mm water). Cardiac output increased to a similar extent (1.0-2.9 L/min) in the 2 groups of animals. In dogs with PA constriction, C_{Cr} was unaltered or increased in all but one dog and C_{PAH} increased in 4 of 5 dogs. Similar changes in renal function occurred in the normal animals. It is concluded that the most consistent differences between the 2 groups of animals in their responses to bleeding were the effects on RAP and Na excretion; RAP increased and Na retention occurred only in dogs with pulmonic stenosis.

123. SOME INPUT-OUTPUT RELATIONSHIPS OF THE COCHLEA. Bruce H. Deatherage* and Hallowell Davis. Central Inst. for the Deaf, St. Louis, Mo.

The 3 electrical responses of guinea-pig cochlea have been measured, at 7000 and 2000 cps, using intracochlear electrodes. The cochlear microphonic (AC) is a linear function of sound pressure level up to about 80 db but reaches a maximum between 95 and 115 db. At 125 db the output is greatly (but reversibly) reduced, although without significant harmonic distortion. The form of the function is very stable although it may be displaced (or truncated) on either axis by various adverse conditions. The summating potential (DC) is proportional to a running time integral (root-mean-square) of the stimulus, as if the cochlea contains a mechanical "detector." Like the cochlear microphonic it is typically linear at low and moderate sound pressure levels and grows less rapidly at higher levels. It does not reach a maximum, however, and although usually much smaller than cochlear microphonic at low intensities, it finally surpasses the latter. Its voltage varies widely, however, with the position of the electrodes along the basilar membrane, the frequency employed and with mild injury. A very labile positive DC response seems to partly obscure a much more stable negative response. Simple action potential (N_1) functions appear only with simple acoustic stimuli like tone bursts with gradual onset. Ideally a very abrupt initial rise in amplitude of N_1 is followed by a slow but monotonic rise (approximately fifth root) up to at least 125 db. Adverse conditions easily elevate the threshold, depress the entire function, and/or introduce great irregularities of increase. (Supported by Office of Naval Research Contract N6onr272(03).)

124. CHANGES IN CELL AND PLASMA VOLUMES PRODUCED BY HEMORRHAGE AND REINFUSION. Stephanie Deavers,* R. A. Huggins, E. L. Smith and R. C. Overton.* Depts. of Pharmacology, Surgery and Physiology, Baylor Univ. College of Medicine, and Dental Branch, Univ. of Texas, Houston.

Sixteen dogs were anesthetized with a combination of morphine and pentobarbital. They were bled until the mean blood pressure was 35.0±2.0 mm Hg. Blood volume was determined by red cells tagged with radioactive Cr^{51} and injected at various times during the experiments. At the end of the bleeding period 9.5 cc/kg of plasma was mobilized but 3.0 cc/kg of the original volume of red cells were trapped. On reinfusion the motilized and 8.2 cc/kg of the dog's original plasma was removed from the circulation or trapped. The final blood volume after reinfusion (69.3 cc/kg) was therefore less than the control blood volume (81.4 cc/kg). Because of the ability of the circulation to adjust the circulating volume by trapping red cells and adding or removing plasma, it is essential to use additional injections of the material to be measured if a change in blood volume is suspected.

125. POSSIBLE QUANTAL NATURE OF SUB-THRESHOLD RESPONSES AT SINGLE NODES OF RANVIER. J. del Castillo* and E. E. Suckling. Dept. of Physiology, State Univ. of New York, Downstate Med. Ctr., New York City.

If a single node of Ranvier of an isolated nerve fiber is stimulated by brief subthreshold pulses of constant strength the resulting non-propagated responses show a marked fluctuation in amplitude (see for details of technique: del Castillo and Stark. J. Physiol. 118:207-215, 1952). In some preparations, visual observation of such responses on a long afterglow CRO-screen suggests the existence of a regularity in their fluctuations. An appreciable proportion of the responses seem to attain certain fixed levels, as if the magnitudes of successive potentials change by steps. This has been investigated by measuring large numbers of local responses and plotting the frequency distribution of their amplitudes. The histograms obtained show clearly, in most cases, a series of peaks separated by regular intervals, which seem to indicate that the recorded responses are multiples of a constant unit. The position of the peaks along the x-axis (amplitudes) is independent of the stimulus strength; successive runs made with different pulse strengths show, in spite of the different averages, peaks at about the same levels. These results could be explained by assuming that the subthreshold response is made up by the addition of a variable number of unit potential changes (equal to 1/100-1/200 of the action potential).

126. BRAIN STIMULATION IN THE MONKEY: TECHNIQUE AND RESULTS (MOTION PICTURE). José M. R. Delgado. Physiology and Psychiatry Depts., Yale Univ. School of Medicine, New Haven, Conn.

Permanent implantation of multilead electrodes in the brain permits study of cerebral function in awake animals. Materials and technique used in construction of surface and depth electrodes and surgery for implantation are shown. Presence of electrodes in the monkey's head apparently does not disturb its behavior. Responses demonstrated are: 1) motor effects: stimulation of area 4 evokes extension of contralateral arm and contralateral rotation of the body. 2) Sensory effects: the monkey scratches its face at each stimulation of the vicinity of the red nucleus. 3) Autonomic effects: coughing, gagging and vomiting are evoked by medullary stimulation; eye movements and nystagmus by tectal area stimulation. 4) Food intake: food ingestion stops and food is rejected when the

neighborhood of the head of the caudate nucleus is stimulated. 5) Conditioning and pain inhibited: a monkey is trained to approach a light to avoid electric shock to the feet; stimulation of septal area inhibits response to both light and shock. 6) Recording of electrical activity: movements of the monkey and simultaneous electrical activity of septal area, motor cortex, and anterior and posterior hippocampus are shown in the film. Stimulation of anterior hippocampus produced automatisms and electrical afterdischarges affecting anterior and posterior hippocampus and septal area, but not motor cortex. Stimulation of motor cortex evokes motor and electrical seizures, first observable in motor area and later in hippocampus. Independence of motor cortex and hippocampus is thus demonstrated. (Supported by Fndn. Fund for Research in Psychiatry and the Office of Naval Research.)

127. SODIUM EXCRETION IN HORMONAL HYPERTENSION. F. Del Greco,* G. M. C. Masson, A. C. Corcoran and I. H. Page. Research Div., Cleveland Clinic, Cleveland, Ohio.

Rats made hypertensive by renal compression, DCA or hydrocortisone treatment present an accelerated excretion of imposed sodium loads (Friedman et al. Circulation Res. 3:297, 1955). It is not known whether this represents a specific renal or extrarenal abnormality, associated in either case with hypertension. The present experiments were designed to study urinary excretory function in anesthetized uninephrectomized rats during mannitol diuresis, untreated (group 1), after development of hypertension provoked by adrenal enucleation (group 2), by treatment with DCA (group 3), cortisone (group 4), and DCA plus cortisone (group 5). Functions studied were urine flow (V), urine and plasma osmolality (P_{Osm}, U_{Osm}) urine and plasma sodium (U_{Na}, P_{Na}), sodium minute output ($U_{Na}V$), renal concentrating mechanism (T^{c}_{H2O}), and glomerular filtration rate (GFR). The relation V to U_{Osm} and T^{c}_{H2O} were in all groups within the limits found in group 1. $U_{Na}V$ was not related to the level of arterial pressure; it was moderately increased in group 5, and showed in all some association with GFR. GFR was slightly reduced in groups 2 and 3, but within normal limits in 4 and 5. It is concluded that hypertensive disease produced by adrenal enucleation, DCA or cortisone does not affect some renal functions such as GFR and T^{c}_{H2O}. In particular, it does not accelerate sodium output during mannitol diuresis.

128. POTENTIAL DIFFERENCES ACROSS THE STOMACH AND DUODENUM OF THE DOG. Warren H. Dennis,* Albert G. Mueller,* Cipriano Canosa* and Warren S. Rehm. Depts. of Physiology and Chemistry, Univ. of Louisville, Louisville, Ky.

In the past, attention has been focused on the electrophysiology of the acid-secreting portion of the stomach and little attention on that of the pyloric portion. The present study is a comparison of the potential differences across the fundus, pylorus and duodenum of the amytalized dog under conditions in which the stomach was not secreting acid. An incision midway between the greater and lesser curvatures was made in the fundus and extended approximately 6 cm into the duodenum along the antimesenteric border. Non-polarizable probe electrodes (5-15 mm diameter) were placed opposite each other across the stomach or duodenal wall. Isotonic saline (solution or agar) made contact with the tissue. Potential differences (PD) were measured with a recording potentiometer. In all of the measurements the serosa was positive to the mucosa in an external circuit. The PD of the fundus was 48-64 mv; of the pylorus, 37-52 mv; and of the duodenum, 2-7 mv. The PD changes rapidly with respect to position at the pyloric-duodenal junction, being 21-39 mv when the electrodes are a few mm on the pyloric side and falling to 5-7 mv a few mm on the duodenal side. (Supported by Army Contract no. DA-49-007-MD-279 and Grant no. G-3503, Natl. Insts. of Health, PHS.)

129. DETERMINANTS OF RENAL TUBULAR TRANSPORT: URATES AND BARBITURATES. Agamemnon Despopoulos (intr. by Willie W. Smith). Natl. Heart Inst., Bethesda, Md.

Uric acid depressed the ability of slices of rabbit kidney cortex to accumulate p-aminohippurate (PAH) without affecting the respiratory rate of the tissue. At an initial urate concentration of 7×10^{-4} M, the accumulation of PAH was depressed by one half. Several derivatives of uric acid were examined in order to correlate molecular structure with inhibitory potency. Barbituric acid, barbital and phenobarbital possessed essentially equivalent potency, but were not as active as uric acid. N-methylation of the barbiturates increased the inhibition, whereas N-ethylation did not. None of the compounds depressed the respiratory rate of the tissues although it was increased by 1-methyl phenobarbital, 1-ethyl phenobarbital and 1, 3-dimethyl barbituric acid. The data may be interpreted as demonstrating a non-ionic interaction between the oxygen radicals of the inhibitor and a component of the PAH transport system producing an apparently non-competitive inhibition of PAH accumulation by slices of rabbit kidney cortex.

130. VASCULAR-EXTRAVASCULAR EXCHANGE OF I^{131} RABBIT GAMMA GLOBULIN IN ORGANS AND TISSUES OF THE RAT. William C. Dewey (intr. by W. F. Bale). Dept. of Radiation Biology, Univ. of Rochester School of Medicine, Rochester, N. Y.

The rate at which injected Cr^{51} tagged rat red cells mixed with circulating red cells was determined. It was assumed that this rate of mixing also measured the rate of vascular mixing of injected I^{131} labeled rabbit gamma globulin, and that subsequent accumulation of I^{131} in organs and tissues represented migration of gamma globulin into extravascular space. Quasi-equilibrium values were reached in which the ratio of I^{131} present extravascularly per unit weight of organ to the value per unit weight of plasma ranged from 0.05-0.10 for muscle, kidney, liver and most other visceral organs to 0.18 for skin and 0.15 for transplanted Walker carcinoma 256. The time required to reach one half of the equilibrium value was less than 5 min for liver, kidney, adrenal and spleen; about 1 hr for heart, lung and small intestine; 4 hr for tumor; and 24 hr for skin and muscle. Calculations indicated that the percentage of the gamma globulin in the plasma which entered the extravascular space of 1 gm of organ or tissue of a 100-gm rat in an hour was as follows: 20% for liver, spleen, kidney, adrenals and ovaries; 5% for heart; 2% for lung and small intestine; 0.2% for muscle and skin; and 0.7% for Walker carcinoma 256. These transfer measurements give insight into rates at which blood-borne antibodies will reach interstitial antigens. (Supported by the Atomic Energy Commission.)

131. PROCESSES CONCERNED IN NET S^{35} ACCUMULATION BY RAT RENAL CORTEX IN VITRO. Ingrith Deyrup. Depts. of Zoology, Barnard College and Columbia Univ., New York City.

Further observations have been made concerning the established process of radiosulfur accumulation by rat renal cortex incubated in media containing $S^{35}O_4$. Measurements were made of the rate of loss of S^{35} after uptake under a variety of conditions, e.g. addition of metabolic inhibitors; and substitution of N_2 for the O_2 atmosphere requisite for optimal accumulation. It was found that, in most cases, cold (1°C) prevented the loss of S35 from renal tissue which always resulted from addition of inhibitors at 37°C. On the other hand, kidney cortex incubated in a medium optimal for S^{35} uptake at 37°C showed a considerable accumulation of the isotope even at 2°C. One interesting consequence of this observation was the suggestion that processes involved in loss of S^{35} from renal tissue in vitro are at least partially distinct from the processes involved in net accumulation of the isotope. The latter may be distinguished from the former by the much greater temperature dependence of loss as compared with uptake of S^{35}. Attempts have been made to determine whether S^{35} exists in the kidney slices in any form other than inorganic sulfate. Methods including extraction with various solvents, precipitation with barium or benzidine, and chromatography have been used, but no evidence has been obtained as yet for the presence of S^{35}-tagged organic compounds in kidney tissue under the conditions of the experiment. (Supported by Research Grant H-2061 from the Natl. Heart Inst., PHS.)

132. POTASSIUM FLUXES IN MYELINATED NERVE. F. P. J. Diecke (intr. by S. R. Bruesch). Dept. of Physiology, Univ. of Tennessee, Memphis.

The influx and outflux of potassium ions in myelinated nerves of frog (Rana pipiens and Rana catesbiana) were measured with radioactive K^{42} during resting state and excitation. The influx during resting state ranges from 10^{-8} to 10^{-9} mol/cm^2/sec, assuming that it takes place primarily at the area of the node of Ranvier. This assumption is justified since the impedance of the total internodal surface is 3-4 times higher than that of the nodal area (Tasaki, 1955). The outflux is of the same order of magnitude. Both values are considerably higher than those reported for unmyelinated nerves (Sepia, Carcinus). They are, however, consistent with the higher conductivity of the nodal area of myelinated fibers. Repetitive excitation at a rate of 60 stimuli/sec or 100 stimuli/sec does not produce any significant changes in potassium influx. The outflux of potassium ions increases by a factor of 2.1 when the nerve is stimulated at 60/sec and 2.6 when stimulated at 100/sec. Thus, an additional potassium outflux of the order of 10^{-11} mol/cm^2/impulse takes place during excitation. The observed ratio of the total outflux at 100 stimuli/sec to the resting outflux differs significantly from the values obtained by Keynes (1951) on unmyelinated nerves of Sepia and Carcinus. These axons show a flux ratio of 9.1 (Sepia) and 20.7 (Carcinus) at the same rate of stimulation. (Supported by PHS Grant B-1268.)

133. RETICULOENDOTHELIAL FUNCTION DURING ALIMENTARY LIPEMIA. N. R. Di Luzio. Div. of Physiology, Univ. of Tennessee, Memphis.

The reticuloendothelial system (RES) may play an important role in the removal of chylomicra from the vascular system. In view of our previous observations of a temporary depression in the removal of colloidal radiogold during an elevation in the plasma level of colloidal thorium dioxide, it became of interest to determine RE function during the peak of alimentary lipemia. Eight dogs previously fasted for 16 hr served as controls. Alimentary lipemia was induced in 12 other fasting dogs by the administration of 4.5 gm/kg of olive oil or lard. Plasma opacities and total lipides were then determined at frequent intervals. When the lipemia reached its maximum value, 0.5 mc of radioactive colloidal gold was injected intravenously and the blood disappearance of the colloid determined. One hour after Au^{198} injection, the animals were killed and liver, lung, spleen and blood were analyzed for radioactivity. The organ distribution and terminal blood concentration of Au^{198} did not deviate from normal in the animals fed either olive oil or lard. Thus, alimentary lipemia did not influence the ultimate fate of the colloid. However, the intravascular half-life of the colloid was significantly decreased in the lipemic animals. This is in marked contrast to the increase in biological half-life of Au^{198} noted subsequent to Thorotrast administration. Out findings suggest that chylomicronemia is capable of physiologically activating the RES. This activation may well account, in part, for the rapid clearing of chylomicra after the ingestion of fatty meals. (Supported in part by PHS grant H2320 and the Memphis Heart Assoc.)

134. HOMEOSTATIC REGULATION OF BODY FLUID VOLUMES: ROLE OF A BLOOD-FLOW-DEPENDENT CONTROL MECHANISM. Ernest L. Dobson. Donner Lab., Univ. of California, Berkeley.

Indirect evidence indicates that under normal isotonic conditions, the adrenal and posterior pituitary release aldosterone and antidiuretic hormone at constant rates. Direct evidence (Ginsburg and Heller 1953) indicates that the rapid rate of destruction of antidiuretic hormone by the liver and kidney is exponential. Aldosterone is probably handled in a manner similar to hydrocortisone which is inactivated exponentially with a half time which is constant over a wide range of concentrations (Peterson et al. 1955). Therefore, we assume the fraction removed from blood with each passage through the liver is independent of hormone concentration and that the exponential rate of inactivation results from constant liver blood flow. Thus, antidiuretic hormone and probably also aldosterone has a removal rate which is a blood-flow-dependent exponential function of time. Furthermore, we have noted that liver blood flow tends to be proportional to blood volume before and after hemorrhage. Constant infusion into blood with exponential removal leads to a constant circulating body content of these hormones, and therefore to an inverse relationship between body fluid volumes and hormone concentration. These hormones are self-diluting so that increased antidiuretic hormone concentration causes water retention and hormone dilution (similarly aldosterone dilutes itself in salt). These three factors, constant infusion, exponential blood-flow-dependent removal and self-diluting hormones provide the framework for a very stable homeostatic mechanism controlling blood and extracellular fluid volume which is dependent on liver and kidney plasma flow.

135. SERUM PROTEIN FRACTIONS AND GLYCOPROTEINS IN PATIENTS WITH INFECTIOUS DISEASE. H. L. Dobson, R. G. Graham, E. M. Yow (intr. by James A. Greene). Dept. of Internal Medicine, Baylor Univ. College of Medicine, and Jefferson Davis Hosp., Houston, Tex.

Serum protein fractions have been studied by means of paper electrophoresis in more than 100 patients with a variety of infectious diseases. In all types of infection the percentage of albumin fell and gamma

globulin increased. In acute bacterial and viral pulmonary infections and in acute bacterial meningeal infections there was a marked increase in alpha 1 and alpha 2 globulins. The bacterial pneumonia patients had the most abnormal patterns but these infections occurred in the summer months. It is felt that these were secondary to other diseases which potentially disturb the electrophoretic patterns. In viral hepatitis the alpha 1 was slightly elevated, alpha 2 normal, and beta was elevated. In mumps alpha 1 was normal and alpha 2 elevated. Excluding hepatitis, the changes in alpha 1 and 2 globulin correlate well with the degree of inflammation as judged clinically. Beta globulin is elevated more in bacterial than in viral infections, with the exception of viral hepatitis. Total glycoprotein has been determined in a series of bacterial pneumonias and found to be increased. The level of glycoprotein falls as the patient recovers. High levels and persistent elevation were felt to be poor prognostia signs. The glycoprotein distribution after electrophoresis was erratic.

136. INCREASE IN GLUCOSE UTILIZATION DURING NEURONAL ACTIVITY. Michel Dolivo and Martin G. Larrabee (intr. by Vernon B. Mountcastle). Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md.

Metabolic measurements at 37°C on sympathetic ganglia excised from rats have previously shown that oxygen consumption accelerates when activity is caused by preganglionic nerve stimulation. Consumption of exogenous glucose also accelerates, but the increase is accounted for as lactate. Therefore the additional oxygen presumably combines with endogenous substates. This agrees with conclusions of other investigators concerning substrate utilization in excised nerve trunk and perfused brain. In recent experiments at 24°C the consumption of glucose also increased during activity, but only a portion was accounted for as lactate, suggesting increased oxidation of glucose. Preliminary experiments with varied pH at both 24° and 37°C suggest that the effect of lowering the temperature may have been caused at least in part by a lowering of pH in the bicarbonate-CO_2 buffer system. Thus ability of nervous tissue to utilize glucose for oxidative generation of additional energy during activity may be significantly influenced by environmental conditions. (Aided by Grant B 702 from the Inst. of Neurological Diseases and Blindness, PHS, and by a fellowship awarded Dr. Dolivo by the Swiss Academy of Medicine.)

137. HUMAN STRESS RESPONSE IN JET AIRCRAFT OPERATIONS. Thaddeus J. Domanski (intr. by O. O. Benson). Dept. of Pathology, School of Aviation Medicine, USAF, Randolph AFB, Texas.

Studies were made on the pre- to postflight blood eosinophil response of: a) two groups of student pilots engaged in different stages of F-86D transition training, b) experienced F-86D pilots engaged in new inflight learning and c) experienced F-94 pilots flying routine training missions. Student pilots operating the F-86D simulator and experienced F-94 radar observers flying routine training missions were also studied. The data include results of a prestress, two postflight, and one postsimulator blood eosinophil counts. The duration of the flights and of the simulator exercises was approximately 1 hr. The first poststress count was drawn at approximately 30 min postflight (or postsimulator); the second at approximately 1.75 hr postflight. Student pilots flying their first mission in the F-86D showed the highest incidence of eosinopenia (85.7%). Minimum such stress response (0-8.3%) occurred in experienced F-94 pilots and in experienced F-94 radar observers flying routine training missions, as well as in student pilots operating the F-86D simulator. An intermediate incidence of eosinopenia (35.7-50.0%) was exhibited by experienced F-86D pilots engaged in new inflight learning and by a select group of student pilots flying in a later stage of the F-86D training program.

138. METABOLISM OF ISOLATED CIRCULAR MUSCLE FROM SMALL INTESTINE OF CAT. H. L. Dorman (intr. by R. E. Johnson). Dept. of Physiology, Univ. of Illinois, Urbana.

Studies on 8 different segments of intact rings of the isolated circular muscle layers, taken equally spaced from the duodenum to the ileocaecal sphincter, showed no consistent gradient in total metabolism. Considerable variation in the rate of oxygen consumption was observed from the different segments in the same cat as well as between different animals. In manometric studies where the gas phase was air, approximately 90% of the glucose used could be accounted for as lactic acid, while CO_2 production was responsible for the remaining 10% of the glucose degradation by the muscle. With 100% oxygen, the glucose converted to lactic acid averaged 80%, whereas with nitrogen the lactic acid production was twice that observed in the oxygen experiments. The addition of mono-iodo-acetic acid to buffered Tyrode solution (ranging from .0005-.0027 M) reduced the lactic acid production to negligible amounts, while the oxygen consumption remained practically unchanged. These observations, and others obtained in preliminary studies with malonic acid as a blocking agent, indicate the presence of a pathway for carbohydrate metabolism that differs from observations reported on striated muscle.

139. CALCULATION OF CONDUCTION VELOCITY OF MYELINATED NERVE FIBER FROM ITS ELECTRIC CONSTANTS. F. T. Dun. Dept. of Physiology, Kirksville College of Osteopathy and Surgery, Kirksville, Mo.

After finding the propagation constant and the characteristic impedance of the node (designated respectively as γ_1 and Z'_o) and those of the myelinated segment (γ_2 and Z''_o), one obtains two sets of auxiliary circuit constants: $A_1 = \text{Cosh } \gamma_1 l_1$; $B_1 = Z'_o \text{ Sinh } \gamma_1 l_1$; $C_1 = (1/Z'_o) \text{ Sinh } \gamma_1 l_1$; $A_2 = \text{Cosh } \gamma_2 l_2$; $B_2 = Z''_o \text{ Sinh } \gamma_2 l_2$; $C_2 = (1/Z''_o) \text{ Sinh } \gamma_2 l_2$; where l_1 = length of node, and l_2 = length of myelinated segment. The generalized circuit constants of an internode $L (= l_2 + l_1$, i.e., a myelinated segment followed by a node) are obtained as follows: $A = A_1 A_2 + C_1 B_2$; $C = A_1 C_2 + C_1 A_2$; $B = A_1 B_2 + B_1 A_2$; $D = A_1 A_2 + B_1 C_2$. When fiber is infinitely long the impedance of the remaining fiber at the end of any internode is $Z_r = [\{(A+D)^2 - 4\}^{1/2} + A - D]/2C$. Let E_s be the spike potential at an active node, I_s and I_r be the resulting voltage and current one internode ahead of it, then, $E_s = I_r (Z_r A + B)$; $E_r = I_r Z_r$. Changing the calculated complex quantity E_r into polar form, one has $|E_r|$, the scalar electrotonic potential drop, and $-\theta°$, its phase lag. The conduction time per internode is $T = \theta°/360°$ msec, the conduction velocity of the fiber is $V = L/T$. Using the electric constants tabulated by Stämpfli (Ergeb. Physiol. 47:137) for frog fiber, and assuming $E_s = 100$ mv, it is found $|E_r| = 47$ mv and $T = 0.05$ msec. After increasing the given capacitances by 50%, which is within the stated limit of propable error, $|E_r| = 44$ mv and $T = 0.07$ msec, agreeing perfectly with actual measurements (Tasaki, Nervous Transmission, 1953, p. 125).

140. PARALLEL DECAY OF ATP AND ACTIVE CATION FLUXES IN STARVED HUMAN ERYTHROCYTES. E. T. Dunham (intr. by Jack Orloff). Natl. Heart Inst., Bethesda, Md.

Freshly drawn red cells were separated from plasma and incubated at 37°C in substrate free media. Suspensions were tagged with K^{42} at frequent intervals up to 36 hr. Measurements included glucose, lactate, Na, K, K^{42} and ATP. After 5 hr, glucose had disappeared and lactate production was reduced to a small fraction of control values (but pyruvate presumably was still being produced at a nearly maximal rate, as has been shown by other work under similar conditions). During the 6th or 7th hr, K influx and cell ATP concurrently began to decline, the latter somewhat more steeply. Concomitantly, cell Na began to rise. When K influx was corrected for the influence of increased cell Na, its decline paralleled that of cell ATP. Both quantities were reduced to one half their initial level at 10 hr. After 24 hr, K influx was 5-10% of initial control level and ATP had virtually disappeared. The influence of strophanthidin on both K influx and ATP degradation in this substrate deprived system has been studied. Whether added initially or during the decline of ATP, strophanthidin (10^{-6} M) markedly reduced the rate of loss of ATP, strongly suggesting an inhibition of ATP dephosphorylation. It produced no obvious effect on glycolysis or onset of ATP decline. The possibility will be considered that K influx and Na efflux are rate-limited by a reaction in which ATP participates, or one which proceeds from ATP and for which ATP provides the most immediate energy reserve.

141. CO_2 RETENTION IN ANIMALS DURING HYPOVENTILATION, WITH ADEQUATE OXYGENATION. Richard H. Earle, John F. Perkins, Jr., Jevrosima Stanimirovitch, Richard Evans, Joan S. Byrne and William E. Adams (intr. by Arno B. Luckhardt). Depts. of Physiology and Surgery, Univ. of Chicago, Chicago, Ill.

This paper represents a quantitative evaluation of the severity of hypercapnea and respiratory acidosis in animals during reduction of tidal volume with constant respiratory rate in the presence of adequate oxygenation, a situation similar to that encountered in open-chest surgery. Total plasma CO_2 was determined by Van Slyke apparatus and blood pH by enclosed glass electrode at 37°C. $PaCO_2$ and plasma bicarbonate concentrations were calculated using the Henderson-Hasselbalch equation. Nine mongrel dogs were anesthetized with Nembutal and given metubine iodide. Arterial oxygen saturation was measured by a Wood-type recording oximeter. The experimental procedure involved maintaining SaO_2 above 80% by increasing PIO_2 while reducing Vt. $PaCO_2$ rose, as predicted from alveolar ventilation, and pH decreased simultaneously. With tidal volume reduced moderately, requiring 272 mm PIO_2 to maintain arterial saturation > 80%, $PaCO_2$ rose from average control value of 38.9 mm-146.9 mm, pH fell from 7.34-6.86. With severe reduction of tidal volume, requiring 681 mm tracheal PO_2 to maintain arterial saturation > 80%, $PaCO_2$ rose to 369 mm, and pH fell to 6.53. Flatness of curve relating pH (abscissae) to HCO_3^- suggests metabolic as well as respiratory acidosis. Above data suggest a means of preventing hypercapnea during chest surgery, adequate ventilation being assured if SaO_2 (measured by oximeter) is kept high while using relatively low concentrations of inspired O_2.

142. INTRACELLULAR POTENTIAL VS TRANSMEMBRANE POTENTIAL OF MUSCLE CELLS. Dexter M. Easton. Div. of Physiology, Florida State Univ., Tallahassee.

The monpolar record of the intracellular action potential at the nerve-muscle junction is depressed in height and prolonged in duration when compared with a response similarly recorded far from the nerve entry in the same muscle fiber. It has been shown that the difference between the 2 records is reduced when the number of adjacent fibers surrounding the impaled fiber is decreased (Easton, Am. J. Physiol. 187:1956). It is now demonstrated that with appropriate recording conditions, the effect of a nearby sink upon the spike configuration is of the same general nature whether the sink originates from nearby fibers or from a cathode placed near the impaled fiber. Recorded from a silent cell, the negative pulse has the appearance of a membrane hyperpolarization, even though the current is outward through the cell membrane. During a response, the negative pulse decreases the recorded spike height. An unpropagated "end-plate-potential" is also distorted by adjacent fiber activity: the record is rounded off at the peak compared with the unaffected response. These observations emphasize that, with respect to records from multicellular preparations, a distinction must be made between 1) the intracellular potential ordinarily measured by means of a microelectrode against an "indifferent" lead, and 2) the actual transmembrane potential measured between 2 electrodes placed respectively just inside and just outside a point on the membrane. (Aided by Grant B-823, from the Natl. Inst. of Health, PHS.)

143. RÔLE OF A SELECTIVE MEMBRANE IN THE GALVANIC SKIN REFLEX. Robert Edelberg, Theodore Greiner* and Neil R. Burch.* Baylor Univ. College of Medicine, Houston, Tex.

Although the vascular and the sweat gland theories of the galvanic skin reflex have both found strong support during an unsettled 65-year controversy, the preponderance of evidence can be interpreted in favor of a membrane hypothesis. This paper offers additional evidence in support of an active membrane in the sweat gland and/or in the epidermal epithelium as the site of the changes in apparent skin resistance following both emotional and physiological stimuli. It has been shown by a system of separate but simultaneous recording from 2 homologous sites that the GSR is profoundly influenced by the nature and concentration of the electrolyte in contact with the skin. Both resistance changes and potential changes are greatly reduced by exposure to electrode paste or low concentrations of NaCl and are greatly potentiated by exposure to $CaCl_2$. In contact with appropriate electrolytes, the membrane shows considerable rectifier properties and the GSR is strongly influenced by the polarity of the current. It has further been demonstrated that any resistance change produced by artificial vascular engorgement is an artefact resulting from the pressure of the engorged segment on the electrode. It is concluded that the GSR depends upon changes in the selective permeability of a negatively charged membrane whose behavior resembles that of nerve membrane. (Work was accomplished under Contract AF18-(603)-79, Office of Scientific Research, USAF.)

144. METABOLIC RATE CHANGES IN NEWBORN DOGS EXPOSED TO COLD. H. E. Ederstrom and Donald McIntyre.* Dept. of Physiology and Pharmacology, Univ. of North Dakota School of Medicine, Grand Forks.

The metabolic rates of conscious dogs at various ages were determined by a closed-chamber oxygen consumption method. In the zone of thermoneutrality (about 30°C) the metabolic rate of dogs under 6 days of age was 30 Cal/sq m/hr. With increasing age the metabolic rate rose slowly, and at 3-4 wk the adult level of 40 Cal/sq m/hr was reached. At air temperatures of 22-24°C animals under 15 days of age doubled their metabolic rates, but rectal temperature nevertheless dropped several degrees. Animals 3-4 wk old maintained rectal temperatures constant in this environment. In a room 3-8°C the metabolic rate of dogs less than 6 days old almost doubled, yet rectal temperature dropped several degrees in 1 hr. In older animals metabolic rates also doubled, but rectal temperatures remained fairly constant, especially in dogs 3 wk or older. The results suggest that the inability of newborn dogs to regulate temperature in a cold environment is not due to low heat production, but rather to high heat loss. (Supported by PHS grant R G-H-4580.)

145. INHIBITORY MECHANISMS OF GAMMA AMINOBUTYRIC ACID ON AN ISOLATED NERVE CELL. Charles Edwards and Stephen W. Kuffler. Wilmer Inst., Johns Hopkins Hosp. and Univ., Baltimore, Md.

Gamma aminobutyric acid (GABA) has been found in brain extracts by Awapara et al., Roberts & Frankel; and Udenfriend (J. Biol. Chem. 187:1950) and apparently plays a role in glutamic acid metabolism (Bessman et al. J. Biol. Chem. 201:1953). It depresses cortical activity (Hayashi et al. Int. Physiol. Cong., 1956) and more recently was found to be part of the "inhibitory factor" of Florey (Bazemore, Elliott & Florey. Nature, Nov. 1956), which inhibits the discharge from isolated nerve cells of the crayfish stretch receptor. This nerve cell possesses synapses on its dendrites formed by an inhibitory axon (Kuffler and Eyzaguirre, J. Gen. Physiol. 39: 1955). The specific inhibitory action of this axon and of applied GABA in concentrations of 10 μg/cc or less shows many similarities. Both drive the activated cell membrane toward its resting potential below the neuron's firing level. There the potential can be held for long periods, thus preventing excitation. This action is confined to the dendrites where stretch deformation sets up the generator potential which leads to conducted impulses. An analysis of the interaction of GABA and of the inhibitory transmitter after nervous stimulation indicates that both produce their effects by increasing the conductance in the synaptic region; K and Cl ions seem principally involved. The mechanism of the block of inhibitory action by picrotoxin will be discussed as well as the action of substances related to GABA.

146. EFFECTS OF MUSCLE SPINDLE DISCHARGE ON MONOSYNAPTIC REFLEX AS REVEALED BY SYNCURINE ADMINISTRATION. E. Eldred, B. Fujimori* and T. Tokizane.* Univ. of California, Los Angeles, and VA Hosp., Long Beach.

Tetramethonium derivatives are known to cause marked acceleration of the discharge from muscle spindles while leaving unaffected that of tendon organs. The present work demonstrates that this is true for both annulospiral and flower spray endings. Thus by this means, the effects of spindular discharge upon central events may be studied. Syncurine given slowly intravenously in quantity sufficient to arrest twitch responses causes a depression of the monosynaptic reflex from the triceps surae of the cat. Injection into the general circulation while the femoral artery of one side is compressed results in depression of the contralateral reflex, but not of that on the side of restricted circulation. Slow injection into the profunda femoris of one side causes a depression of the monosynaptic of that side, but little or no effect on the contralateral side. It is concluded that at least one of the spindle afferent modalities has an inhibitory effect upon the monosynaptic reflex.

147. ASSAY OF LUTEINIZING HORMONE. Stanley Ellis and Jean Porter (intr. by Jane A. Russell). Dept. of Biochemistry, Emory Univ., Ga.

Ovarian hyperemia would appear to offer a rapid and sensitive assay for the estimation of luteinizing hormone (LH) if it could be put on a quantitative basis. Studies toward this end have been carried out in immature rats utilizing radio-iodinated human serum albumin (RISA) as an indicator of ovarian hyperemia. The injection of LH into the tail vein was followed by a tail vein injection of RISA at various intervals thereafter. After allowing 10 min for equilibration, the ovaries were removed and the radioactivity measured with a scintillation detector. A maximal increase in the RISA volume of the ovaries of about 110% is reached at 2 hr following LH, after which a decline occurs. Using 2 hr as a standard time for development of hyperemia, a linear relationship between RISA uptake and dose of LH was established. Linearity was evident between 1-8 μg of LH. The results indicate that estimation of LH potency is feasible by this method. Neither lactogenic nor follicle-stimulating hormones interfere.

148. EFFECT OF DISCONTINUOUS TREATMENT OF THE NORWAY RAT WITH GROWTH HORMONE. J. D. Emerson and G. M. Emerson (intr. by T. W. Robinson). Dept. of Physiology, Univ. of Alabama Med. Ctr., Birmingham.

Daily injection of the Norway rat with anterior pituitary growth hormone leads to an initial period of major growth followed by reduced growth, and eventual growth stasis (plateauing) even though treatment is continued at the same dose level either per animal or per unit weight. This plateauing appears to be independent of purity of the growth hormone preparation or age of the rats. Identical plateauing occurs on treatment with extracts of rat pituitaries. At a high dose level, the rats may attain at least 2.5 times adult size prior to plateauing. In this experiment 16 7-month-old female rats were given daily injections of a pH 10.5 pituitary extract equivalent to 3.2 mg growth hormone/rat/day. After 136 days of treatment there was a mean gain of 187.3 ± 13.1* gm and the animals had reached a plateau (2.1 ± 4.8 gm gain over the controls in a 16-day period). Eight of the rats were then put on 4 alternating periods of 16 days of no treatment and 16 days on treatment, while the remaining 8 rats received continuous daily injections as previously and remained plateaued. During each of the 16-day no-treatment periods there was a mean loss of approximately 60 gm which was regained (55.0 ± 11.8-62.0 ± 7.4 gm) during the 16-day retreatment period, compared to a gain of 63.9 ± 1.7 gm for the initial 16 days of treatment.

149. COMPLIANCE OF ARTERIES, VEINS AND CAPILLARIES IN ISOLATED RABBIT LUNGS. Joseph Engelberg* and Arthur B. DuBois. Dept. of Physiology and Pharmacology, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

Using a combination of techniques, we have measured the pressure-volume relationship for the whole vascular bed and for the arterial, venous and capillary

components separately, in isolated Krebs-Ringer perfused rabbit lungs. The P - V relationship for the whole system was measured directly, by observing the pressure response to known volume increments, and by Evans Blue space measurements. The P - V relationship of the arteries or veins having diameters larger than about 30 μ was measured directly after embolization of the arterioles or venules with lycopodium spores. Another technique measured the P - V relationship of the arterial vessels down to, but not including, the capillaries (those involved in gas exchange). Here arterial inflow, arterial pressure and capillary flow, measured plethysmographically by the uptake of N_2O from alveolar gas (Lee and DuBois. J. Clin. Invest. 34:1380, 1955), were measured simultaneously. The volume change in the arterial system was then calculated, by subtracting the volume following into the capillaries from the volume introduced into the arteries. This measurement was repeated for the venous system using retrograde perfusion. The fluid flow resistances during retrograde and forward perfusion were similar. In 4 rabbits (mean weight 4.1 kg) using the N_2O uptake method, the following approximate mean compliance ($\Delta V/\Delta P$) values in ml/cm H_2O were obtained: pulmonary arteries + capillaries + venis = 0.73; left atrium = 0.12; arterial system = 0.16; venous = 0.42; "capillaries" (by subtraction) = 0.15.

150. EFFECT OF WEIGHT REDUCTION COMPOSITION AND DENSITY OF HUMAN ADIPOSE TISSUE. Cecil Entenman, Albert R. Behnke, Nell S. Ayres* and William H. Goldwater.* U. S. Naval Radiological Defense Lab., San Francisco, Calif.

In order to know more accurately the nature of the material lost during weight reduction, calculations of the body fat, water and lean body mass have been made for 2 human subjects before and after weight reduction by a standard controlled diet. Densities of 0.94-0.96 were found for the material lost during body weight reductions of 12-15%. Since fat has a density of 0.90 the results indicate a loss of denser body constituents. However, chemical determination made on biopsy samples of subcutaneous adipose tissue removed before and after weight loss in each individual indicate that these additional substances did not arise wholly from adipose tissue. The adipose tissue samples were analyzed for fat, water, dry residue and deoxyribonucleic acid (DNA). Based on their proportions in the adipose tissue, fat decreased 10-20%, while water and residue rose almost two-fold. Related to the dry, defatted residue, however, fat decreased to one half its original level, while water dropped only slightly. The DNA content per residue weight remained constant through the weight-reduction period. Histologic studies indicate about a 50% increase in fat cells per unit field after dieting, which is of the same order of magnitude expected from the relative decrease of depot fat. The densities of the excised adipose tissue were greater following weight reduction indicating a loss of low density material, presumably fat. Densities calculated from the chemical composition agreed closely with densities determined at room temperature on the freshly excised tissue.

151. POLYETHENOID ACIDS AS ENERGY SOURCES IN RESTING AND EXERCISED RAT. John D. Evans and Donald E. Parlee.* Physiology Dept., Temple Univ. School of Medicine, Philadelphia, Pa.

White male rats of comparable size and age, under conditions of constant temperature and adequate water supply, were fed commercial rat food in carefully weighed amounts sufficient to keep them at constant weights. Feces from each rat were also carefully collected and weighed. Spectrophotometric analyses of the fatty acids from food and feces provided quantitative data on the absolute amounts of polyunsaturated acids ingested and excreted during the experimental period. The data show the composition of food and fecal fat to be greatly different and clearly demonstrate that the major fraction of consumed linoleic acid is either metabolized or stored. Quantitative information was also obtained for the other polyethenoid acids. Two animals were exercised in activity cages under circumstances that forced them to run known distances. Total body fat from control and exercised rats was extracted and the fatty acids obtained were analyzed spectrophotometrically. Comparison of the total body fatty acids from control and experimental animals showed that linoleic acid constituted a smaller percentage of the fatty acid in exercised than in control animals, whereas arachidonic acid constituted a larger fraction. Analyses also showed that the amount of total cholesterol in total body lipides was not affected by exercise. From the balance sheets obtained in this study we conclude that linoleic acid is an important source of energy for metabolism and exercise.

152. MUSCLE OXYGEN CONSUMPTION AND ISOTONIC WORK IN THE DOG. John T. Fales,* S. Richard Heisey* and Kenneth L. Zierler. Dept. of Environmental Medicine and Medicine, Johns Hopkins Univ., Baltimore, Md.

Studies designed to determine the relation between work and muscle oxygen consumption ($\dot{Q}O_2$ = total oxygen uptake minus resting) were performed on the isolated gastrocnemius-plantaris muscle of the intact dog. Shortening was produced by supramaximal volleys to the sciatic; duration of stimulus adjusted to permit no more than 95% of maximum shortening. When work varied by varying the number of tetanic contractions at constant load, $\dot{Q}O_2$ seemed to be linearly related to work. When work varied only by varying load, for small loads $\dot{Q}O_2$ rose sharply with increasing work. However for work between 0.3 and 1.5 kg-cm/gm of muscle $\dot{Q}O_2$ increased only slightly if at all. Over this range of work, shortening and the time required to reach 95% of maximum shortening were nearly constant. Thus, despite 5-fold variation in work, the same number of test stimuli were delivered. By varying frequency of stimulation and by varying duration of stimulation at constant load to permit variations from about 25-95% of maximum shortening, it was found that the relation between $\dot{Q}O_2$ and work was best described as dependent upon the number of impulses delivered. $\dot{Q}O_2$ per impulse decreased exponentially with the number of impulses. Each impulse appears to explode the consumption of a fixed fraction of oxidizable substrate remaining in muscle at the time of its arrival. (Supported by Office of Naval Research and grants from Natl. Insts. of Health.)

153. RESPONSES OF HAMSTERS DURING FIRST TWO WEEKS OF COLD EXPOSURE. Richard L. Farrand* and G. Edgar Folk, Jr. Dept. of Physiology, State Univ. of Iowa, Iowa City.

The responses of hamsters were determined at 25° and 5°C and the results compared. The cold exposed animals were limited to no more than 15 days of exposure. There was a slight but definite decrease in body weight during the first 2 days of cold exposure, reaching a maximum loss of approximately 20% at about the 6th day. No further alteration in weight

loss occurred during the remainder of the cold exposure. Food consumption increased progressively so that by the 6th day they were consuming twice the normal amount. Water intake decreased during the first 2 days, then progressively increased until by the 6th day it had attained a level of some 60% above normal and remained there. As anticipated, total oxygen consumption increased markedly on the first day and remained at about this level during the duration of the experiment. The rectal temperatures were unaltered as a consequence of the cold exposure. The heart and kidney weights of the animals exposed to the cold were slightly greater than the control animals; the major portion of this weight change appeared after the first day of exposure. Skin weight decreased and no change was observed for splenic weights. Estimates of plasma, extracellular and total fluid volumes were made on the final day of exposure. (Research aided by Natl. Science Fndn.)

154. EFFECT OF ACETYLPHENYLHYDRAZINE ON MYOGLOBIN CONCENTRATION. Willard R. Faulkner* and Frank R. Blood. Dept. of Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

The administration of acetylphenylhydrazine produces hemolytic anemia. The compound appears to act by damaging the mature erythrocytes, rendering them highly susceptible to phagocytic destruction by cells of the reticulo-endothelial system. Also, acetylphenylhydrazine has been shown to cause a marked reduction in cytochrome c concentration of muscular tissues. This had led to the suggestion that there is a common biosynthetic origin for all heme derivatives. Since myoglobin is a heme derivative, it seemed probable that its concentration would be reduced after acetylphenylhydrazine administration. To test this, female rats of the Sprague-Dawley strain were paired and acetylphenylhydrazine administered daily to one member of each pair by subcutaneous injection. The paired control was given a corresponding quantity of physiological saline. After 94 days the treated animals had developed marked anemia; all rats were sacrificed for myoglobin assay of their skeletal muscle. The mean for the treated animals was 0.87 ± 0.03 mg/gm and for the controls 1.30 ± 0.06 mg/gm. So it appears that acetylphenylhydrazine does affect porphyrin synthesis.

155. REWARMING FOLLOWING PROLONGED HYPOTHERMIA. E. J. Fedor, Clem Russ, S. H. Lee and Bernard Fisher (intr. by P. L. McLain). Dept. of Surgery, Univ. of Pittsburgh, Pittsburgh, Pa.

This laboratory has demonstrated that as hypothermia progresses the more complex become the physiologic alterations in such parameters as pulse, blood pressure, venous pressure, hematocrit, clotting and prothrombin times, electrolytes, cardiac output etc. A systematic evaluation of the effects of rewarming on these changes has been undertaken with the express purpose of determining how complete and at what rate these changes revert to normal following rewarming. In animals maintained at 23°C for 8 hr and then rewarmed there was a prompt return to normothermic pre-cooled levels of venous pressure, pulse rate, clotting time, blood pH, plasma specific gravity, blood sugar, serum sodium and calcium. Blood pressure and prothrombin time lags. Hematocrit promptly returns to and falls below the pre-cooled value and serum potassium shows an increase. The prompt readjustment of these values following rewarming is compatible with the survival rate of 100%, following 8 hr of hypothermia and rewarming. Following 12 hr of cooling the return to normal is not as prompt and mortality rate is approximately 50%. Observations on cardiac output indicate that even after many hours of rewarming following prolonged hypothermia there is a failure in the return of cardiac index to normal pre-cooled levels while oxygen consumption rapidly becomes normal. Oxygen consumption, for example, after 12 hr of hypothermia at 23°C and just before rewarming, was 2.9 ml/kg/mm. After 1, 5, 9, 13 and 24 hr of rewarming, values were 11.2, 9.4, 9.0, 9.7, 9.1 ml/kg/mm., respectively. (Aided by Research Grant H-2065(C) from the Natl. Insts. of Health, PHS, and Dept. of the Army Grant DA-49-007-MD-715.)

156. EFFECT OF HYPOXEMIA ON CORONARY FLOW AND CARDIAC OXYGEN CONSUMPTION. H. Feinberg,* A. Gerola* and L. N. Katz. Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.

The effect of increasing severity of hypoxemia on coronary flow and myocardial oxygen metabolism was studied at different levels of cardiac work in the open-chest anesthetized dog. Total coronary flow was measured by the methods developed in this laboratory. Cardiac work was altered by increasing the blood pressure at constant cardiac output. Hypoxemia was induced by positive pressure breathing of low oxygen tension gases of various concentrations. Coronary flow increased with increasing severity of hypoxemia at the same level of cardiac work. Arterial oxygen saturation diminished at a greater rate than the venous oxygen, hence the A-V oxygen difference decreased with hypoxemia. The A-V oxygen difference remained independent of the cardiac work and rate throughout the entire range of arterial oxygen saturation studied. Thus, at any given arterial oxygen saturation coronary flow was found to be the principal variable in the calculation of oxygen consumption over a wide range of work and rate. Oxygen consumption at isowork levels remained relatively constant over a large range of diminished arterial oxygen saturation but declined slightly in the lower range.

157. SYNTHESIS OF LIPIDS FROM RADIOACTIVE METHYLMALONATE BY ADIPOSE TISSUE SLICES. D. D. Feller and E. Feist.* VA Hosp. and Univ. of Washington School of Medicine, Seattle.

Inguinal adipose tissue obtained from male Swiss mice fed an adequate diet ad lib was sliced and incubated in the presence of bicarbonate-C-14, propionate-1-C-14, and methylmalonate (carboxyl-C-14). Saponifiable lipids were recovered by standard procedures and C-14 content determined in this as well as in the CO_2 fraction. Liver slices were used as a control tissue. Results demonstrate that surviving adipose tissue is capable of synthesizing fatty acids from methylmalonate to an extent 100-200 times greater than liver. These observations are comparable to those found when propionate-1-C-14 was used as a substrate, although the net conversion of propionate to fatty acids by both tissues was about 4 times greater than for methylmalonate. Addition of carrier methylmalonate at 0.01 M concentration reduced conversion of propionate-1-C-14 to fatty acids to a value one tenth that of the control and conversion to CO_2 to a value one half that of control. This observation led to an experiment whereby bicarbonate-1-C-14 was incubated in the presence of 0.01 M propionate. No measurable amounts of C-14 was recovered in the fatty acid fraction. These results show that methylmalonate is a precursor in the biosynthesis of lipids

in adipose tissue. The data indicate that methylmalonate may be an intermediate in the path of propionate to fatty acid and, if so, it is formed by a mechanism not utilizing condensation of bicarbonate with propionate as a major path. (Supported by a grant from the American Cancer Soc.)

158. METABOLISM OF AN ALBUMIN-PALMITATE-C^{14} COMPLEX BY DIABETIC RAT LIVER SLICES. J. M. Felts (intr. by A. Canzanelli). Dept. of Physiology, Tufts Univ. School of Medicine, Boston, Mass.

Albumin is known to form reversible complexes with free fatty acids and, also, may function as an acceptor for free fatty acids when triglycerides are acted upon by plasma clearing factor. It would appear likely, therefore, that an albumin-fatty acid complex could serve as an active tissue substrate for the transfer of fatty acids into the cell. A study was made of such a complex prepared from serum albumin and palmitate-1-C^{14}. This substrate was found to be extremely active metabolically when incubated with liver slices. A large fraction of the C^{14} was found in the triglyceride fraction of the slice. This complex was used to study fatty acid esterification in liver slices from diabetic rats. It is well established that there is a block in lipogenesis from c-2 units derived from acetate and other substrates. The present experiments indicate that there is no consistent defect present in the esterification of palmitic acid in diabetic liver slices. When a depression is noted in esterification, it is not of the same order of magnitude as the defect found in lipogenesis from acetate. This evidence indicates that the availability of glycerol or a failure in esterification are not the cause of the failure of lipogenesis found in the diabetic liver.

159. PROPERTIES OF PURIFIED PREPARATIONS OF PLATELET COFACTOR I (ANTIHEMOPHILIC FACTOR). Richard L. Fenichel (intr. by F. Morin). Dept. of Physiology and Pharmacology, Wayne State Univ. College of Medicine, Detroit, Mich.

Methods have been developed for obtaining very active concentrates of platelet cofactor I from human plasma and bovine plasma. In collaboration with Walter H. Seegers and Ricardo H. Landaburu, the preparations obtained from bovine sources have been studied extensively. The platelet cofactor I functions with purified platelet factor 3, Ac-globulin, and calcium ions in the conversion of purified prothrombin to thrombin. The thrombin yield becomes incomplete when the quantity of platelet cofactor I is reduced; and within a certain range of concentration, the thrombin yield reflects the quantity of platelet cofactor I activity. This is the basis for the quantitative determination of platelet cofactor I activity. Within the range pH 5.5-7.5, the activity is stable for long periods of time. In 50% glycerol solution, no activity was lost during one-half year of storage in a deep freeze. If the acidity is greater than pH 4.5, the activity is rapidly destroyed. The isoelectric point is pH 6.4 as determined by electrophoresis and a single peak is obtained with the moving boundary method. Between 30° and 35°C, the activity is rapidly destroyed.

160. ACTION OF SEROTONIN ON MOTILITY OF ANEURAL SMOOTH MUSCLE. John Ferguson. Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Nebr.

Preparations of the amnion of the developing chick were made and each was suspended as a muscle strip in Sollmann-Rademaekers' solution. Rhythmic and tonic changes were recorded. These structures contain muscle tissue but are devoid of nervous elements of any description. They manifested spontaneous motility in about 80% of the preparations. Generally, a concentration of 2×10^{-8} of serotonin creatinine sulfate increased motility in a rhythmic preparation; a quiescent strip required a higher concentration. Low or moderate doses of serotonin caused responses which were reproducible without any sign of tachyphylaxis; at high concentration, the responses were variable and difficult to evaluate. At no concentration did the drug depress activity. The threshold dose required to enhance motility in excised rabbit's intestine did not differ materially from that required to augment motility in amniotic preparations. Atropine sulfate when added before or after decreased the effectiveness of serotonin but to a lesser extent than it inhibited the stimulating effect of acetylcholine on this muscle. Hexamethonium chloride in any concentration failed to depress spontaneous motility in this muscle or to inhibit the effect of serotonin on its motility. The addition of a small amount of sodium chloride, sodium sulfate, creatinine, glucose or hydrochloric acid to the bath failed to modify significantly spontaneous motility in fresh preparations. (Aided by Nebraska Heart Assoc.)

161. EFFECT OF SHALLOW AND DEEP BREATHING ON PULMONARY COMPLIANCE. B. G. Ferris, Jr. and D. Pollard.* Dept. of Physiology, Harvard School of Public Health, Boston, Mass.

Previous studies have shown that the lungs and "thorax" of severely paralyzed poliomyelitis patients are less compliant than normal. Since the weakened muscles are unable to expand the lungs to their former inspiratory capacity it was thought that expansion by mechanical means might improve pulmonary compliance. The compliance in the tidal volume range can be improved nearly 30% by deep breaths with a pressure difference across the lungs of 20-22 cm H_2O. In one patient, for example, control values were 0.036 l/cm H_2O and after stretching were 0.045 l/cm H_2O. Two hours later compliance had fallen to 0.040 l/cm H_2O. Similar studies were done on normal subjects during spontaneous respiration. The pulmonary compliance was measured in the tidal volume range while the subject was supine. During the period of shallow breathing respiratory excursions were monitored by means of a sensitive pneumograph. There was a 25% reduction in the pulmonary compliance in a half hour. This change was reversible and one or two deep breaths with a pressure difference across the lungs of 19-21 cm H_2O could return the pulmonary compliance to its former value. Additional deep breaths did not improve the compliance further. There is thus a progressive but reversible fall in pulmonary compliance associated with shallow breathing in the supine position.

162. FRACTIONATION OF NEUTRAL SALT-SOLUBLE COLLAGEN, USING TEMPERATURE DEPENDENCE OF SOLUBILITY. J. H. Fessler (intr. by Jerome Gross). Lovett Mem. Labs., Massachusetts Gen. Hosp., Boston.

Preparations of neutral salt soluble collagen and their transformation into typical collagen fibres were separately described by Gross, Highberger and Schmitt, and by Jackson and Fessler. Jackson and Fessler's preparation (Nature 176:69, 1955) is now shown to contain 3 fractions whose properties vary differently with temperature. When a solution of this material is warmed to 37°C a gel composed of fine fibres is formed, but some 16% of the protein is not precipitated and this soluble component is called

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fraction B. On standing a gelled sample in the refrigerator, some 70% of the gel dissolves within 2 days, and this portion is called fraction A. The remaining 30% of gel is called fraction C, and dissolves only very slowly in the cold. Fraction A can be repeatedly gelled and redissolved by varying the temperature. The proportions of the 3 fractions are independent of concentration and pH (6.5-7.4). The behavior of the 3 separate fractions and the initial material differed only little in: the ultracentrifuge, the Couette viscometer and the birefringence apparatus. Particles with weights of the order of 500,000 and several thousand Angstrom units long were indicated. Probably the 3 fractions contain closely similar material and while they may not correspond to separate entities in the animal body, their possible role in fibrogenesis is being investigated. (Supported by Med. Research Council, London for work done at the Dept. of Biochemistry, Oxford.)

163. QUANTITATIVE ASPECTS OF ERYTHROPOESIS. Clement A. Finch, Dennis M. Donohue,* Robert H. Reiff* and Mary Lu Hanson.* Dept. of Medicine and Pathology, Univ. of Washington, Seattle.

Methods have been devised for enumerating the total red cell procursers within the marrow. This involves cell counts of a portion of marrow and the relation of this to total marrow through respective ratios of porphyrin, marrow heme and marrow-encorporated Fe^{59}. The number of circulating erythrocytes and reticulocytes were measured with standard counting and Cr^{51} or P_{32} volume techniques. The erythron was arbitrarily divided into 4 components: nucleated erythrocytes, marrow reticulocytes, circulating reticulocytes and mature circulation erythrocytes. In the rabbit the relative numbers of these 4-cell populations was found to be 7, 6, 32, 1000 respectively. By employing radio-iron to measure time of maturation in marrow (16 hr) and survival in peripheral blood (40 days), it was possible to make calculations of maturation time of marrow and blood reticulocyte, and of rate of multiplication of nucleated erythrocytes. The maturation time of marrow reticulocytes has been established at 6 hr and of circulating reticulocyte at 32 hr. The nucleated red cell mass doubles in number every 8 hr. Similar studies have been carried out in other animals, including rat, dog, monkey and man. In addition to the absolute numbers of erythroid forms, the relative rates of reproduction of nucleated red cells and times of maturation of erythrocytes of these various species will be compared.

164. AERODYNAMICS OF VOCAL FOLD VIBRATION DURING PHONATION IN MAN. B. Raymond Fink and F. Kirschner (intr. by L. J. Cizek). Dept. of Anesthesiology, Columbia Univ., and General Electric Corp., New York City.

The vibration of the human vocal folds during phonation was studied by detailed examination of the Bell Telephone high-speed motion picture and by antero-posterior tomograms. Viewed from above, when vibratory movement begins, the arytenoids are in contact and the medial edges of the vocal folds appear concave. The glottis in the starting position thus presents a biconvex outline. The first change observed is that the glottis becomes narrower, though still convex. The glottis then widens to beyond the starting width, followed by a phase of narrowing, and so on until a steady amplitude of vibration is attained. Steady state vibration is established after about 3 cycles. The vibration is not sinusoidal but is rather a saw-tooth wave with a sharp wave-front and a slow decay. The axis of oscillation is not in the midline but approximately in the starting position of the vocal folds. The concave curvature of the vocal folds at the onset of phonation enables the larynx to utilize the dynamic pressure change produced by air flow through the glottis. The effect is that the amplitude of oscillation of the vocal folds necessary for a given sound volume is much smaller than previously assumed, and that the force of impact between the vocal folds is minimized. Antero-posterior tomograms show that during phonation the vocal and also the vestibular folds assume a curved shape resembling the exponential horn of a loudspeaker, constituting an acoustical adaptor between two different cross-sections of an air passage. In phonation of low volume the vocal folds make contact only at their superomedial edges; the infraglottic surfaces of the vocal folds diverge immediately below this level. Experiments with rubber membranes indicate that the angle of divergence affects the pressure differential necessary for the maintenance of vibration; a sharply smaller differential sufficed when the angle of divergence of the membranes was in the range 45-58°.

165. SPLANCHNIC, HEPATIC WEDGE, AND ARTERIAL BLOOD PRESSURES FOLLOWING PORTACAVAL SHUNT. Bernard Fisher, E. J. Fedor* and S. H. Lee.* Dept. of Surgery, Section of Exptl. Surgery, Univ. of Pittsburgh, Pittsburgh, Pa.

Measurement of splanchnic, hepatic wedge and arterial blood pressures were made pre-shunt in normal unanesthetized dogs with catheters previously inserted into a splenic vein, during shunt (anesthetized) and for varying periods of time after shunt up to 1 month. In animals upon whom large end-to-side fistulae were performed, preoperative portal pressures averaged 10 cm of saline. Immediately following the operation pressures were 12.7 cm and within 1 hr after were 15 cm. Post-operative unanesthetized pressures averaged 12.4 cm. Following side-to-side anastomosis pressures averaged 9.9 cm in the post-operative period. Pre-operative portal pressures in this group of animals was 10.9 cm. All shunts were performed so that no narrowing of the portal vein occurred and so that the orifice of the shunt was larger than the normal circumference of the portal vein. It is to be concluded that portacaval shunt in the normal animal does not appreciably reduce the portal-bed pressure. Hepatic vein wedge pressures immediately following end-to-side portacaval shunt decreased 39% dropping from 12.7-7.8 cm, but in the later post-operative period pressures were 9.6 cm, a decrease of only 24%. This would seem to indicate that the arterial component of the wedge pressure is perhaps greater than heretofore described. Upon clamping of the portal vein to perform an Eck fistula the arterial blood pressure consistently drops to near shock levels and upon completion of the shunt with removal of clamps does not return to the pre-shunt level for several hours. In sham-operated animals, identical to the Eck fistula dog except for the deviation of portal blood, systemic blood pressure returned to normal immediately upon release of the portal vein clamp. (Supported in part by Grant A-369(C3) from the Natl. Insts. of Health. PHS.)

166. GLUCOSE TOLERANCE CURVES IN PARABIOTIC RATS. David G. Fleming and Mary A. Nugent (intr. by O. O. Stoland). Dept. of Physiology, Univ. of Kansas, Lawrence.

Glucose tolerance responses of parabiotic rats were compared with those of single rats. Animals,

parabiosed at 21 days, were held until their combined weights reached approximately 500 gm. One member of each pair was stomach-tubed with 2 cc of a test solution, containing either 1.0 or 2.0 gm of glucose, or saline for controls. Tail puncture blood was taken from both animals before stomach-tubing one of the pair, then 1/2, 1, and 2 hr afterward. Blood sugars were determined by the anthrone colorimetric method. Single rats, fed 1.0 gm of glucose, were checked similarly. Ten parabiotics, given 1.0 gm of glucose, showed the following values: fasting - 85.5 mg%, 30 min- 130 mg%, 60 min- 118 mg% and 120 min- 100 mg%. Their partners' blood sugar did not rise, but declined slightly from fasting levels. As expected, control blood sugars fell steadily. Eight parabiotics, given 2.0 gm of glucose, responded as follows: fasting- 81 mg%, 30 min- 167.5 mg%, 60 min- 142.5 mg%, and 120 min- 131.5 mg%. Their partners showed no rise in blood sugar, but maintained fasting levels. Blood sugars on 250-gm single rats, fed 1.0 gm of glucose, showed the following values: fasting- 92 mg%, 30 min- 125.5 mg%, 60 min- 124.3 mg%, and 120 min- 113 mg%. Orally administered glucose given to 1 member of a parabiotic pair does not cross the parabiotic barrier rapidly enough to exceed the rate of utilization by its partner. Therefore, the blood sugar of the unfed partner remains essentially constant.

167. RADIOACTIVE IODIDE IN MUCOUS AND PEPTIC GLANDS OF THE RAT STOMACH USING A DOUBLE-CHAMBERED DIFFUSION APPARATUS. William C. Foster, Bernard P. Lewis* and Franklin H. Paul.* Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.

Earlier studies revealed that rats injected with radioactive iodide formed more tissue protein-bound radioactive iodide in the peptic gland portion of the stomach than mucous gland portion. When stomachs of uninjected rats were suspended in a Tyrode's Solution bath containing radioactive iodide for 2 hr at 37°C the reverse occurred. In the present studies the stomachs of adult Wistar-strain rats were quickly extirpated, the 2 glandular portions of the stomach separated and each portion mounted between the chambers of a double-cell diffusion apparatus. Two microcuries of radioactive iodide in Tyrode's solution were placed in either chamber, thereby placing the iodide in contact with the serous or glandular surface of the stomach. The apparatus was then placed in a water bath at 37°C, and a CO_2 - O_2 mixture bubbled into the chambers. After a 2-hr uptake period, the plugs of tissue exposed the radioactivity were cut out, weighed, and counts/min/tissue plug determined. The weight ratio of mucous gland plug to peptic gland averaged 1:3, whereas the uptake of radioactive iodide by these tissues averaged only 1:1.5 when the radioactivity was on the serous side. When the glandular or lumen surface was exposed to radioactivity the ratio increased 10-fold. The major factors affecting the ratios were thyroidectomy, adrenalectomy and pH. The radioactivity of the solutions in the 2 chambers were determined at half-hour intervals during each experiment. (Supported by a grant from the Natl. Heart Inst., PHS.)

168. A TRICARBOCYANINE DYE FOR CONTINUOUS RECORDING OF DILUTION CURVES IN WHOLE BLOOD INDEPENDENT OF VARIATIONS IN BLOOD OXYGEN SATURATION. I. J. Fox, L. G. S. Brooker, D. W. Heseltine and H. E. Essex (intr. by F. C. Mann). Mayo Fndn. and Mayo Clinic, Rochester, Minn., and Eastman Kodak Research Labs., Rochester, N.Y.

A serious disadvantage of oximeters and densitometers in recording indicator-dilution curves in whole blood is their inability to distinguish between variations in concentration of oxy- and reduced hemoglobin and the indicators used (usually T-1824). A suitable indicator with high spectral absorption at a wave length equally absorbed by oxy- and reduced hemoglobin (e.g. 800 mμ) would circumvent this disadvantage, permitting recording of dilution curves on the venous side of the circulation and in arterial blood, despite variations in oxygen saturation. A water-soluble tricarbocyanine dye (dye II) having this characteristic has been developed. After rapid mixing of this dye and blood, the spectral transmission of the mixture stabilizes more quickly than with T-1824 (< 10 sec). Sudden injections of 0.1 mg/kg cause no detectable reaction in dogs, and produce dilution curves giving values for cardiac output similar to those calculated from near-simultaneous T-1824 or indigo carmine curves (± 11%) and by the Fick method (± 14%). The dye is rapidly lost from the blood; 10 min after intravenous injection, the apparent dye II-space is nearly doubled from its 2-min value, compared to a 15% increase for T-1824. No untoward effects followed 10 mg/kg given intravenously (100 times the requirement for a dilution curve).

169. "SITE OF ORIGIN" OF REPETITIVE RESPONSES DURING VERATRINE AFTER-DISCHARGE. George B. Frank (intr. by B. Delisle Burns). Dept. of Physiology, McGill Univ., Montreal, Canada.

It was found that a veratrine after-discharge was produced in frog's skeletal muscle when a sufficient gradient of membrane potential per unit length was established following an action potential. This effect was usually produced by treating only a portion of the fiber's surface with veratrine (Burns et al. Brit. J. Pharmacol. 10:363, 1955). The membrane potential changes associated with the conditions leading up to the start of the after-discharge were studied with inside-the-cell microelectrodes. With external electrodes the "site of origin" of the repetitive responses was located in the junctional region between treated and untreated lengths of fiber and seemed to be on the treated side of the junction. The exact site of origin was located by inserting 2 inside-the-cell microelectrodes into a single fiber and recording the latencies of the after-discharges. In this way their origin was located approximately 1 space constant (λ) from the junction on the veratrine-treated side.

170. PRESYNAPTIC AND POSTSYNAPTIC INHIBITION OF MONOSYNAPTIC REFLEXES. K. Frank and M. G. F. Fuortes. Lab. of Neurophysiology and Ophthalmology Branch, Natl. Inst. of Neurological Diseases and Blindness, Bethesda, Md.

Brock, Coombs and Eccles (J. Physiol. 117:431, 1952; and 130:326, 1955) reported that inhibition of monosynaptic reflexes occurs by hyperpolarization of the motoneurone membrane produced by selective increase of the membrane permeability to certain ions. The same motoneurone hyperpolarization has been recorded in some but not in all cases of inhibition of monosynaptic reflexes. Hamstring motoneurones were shown to be hyperpolarized by single inhibitory volleys from quadriceps nerve with a total latency of 2 msec. The time course of this hyperpolarization was similar to that of the depolarization produced in the same motoneurones by a shock to the hamstring nerve. This hyperpolarization is accompanied by decreased electrical excitability as measured by direct stimulation through the impaling microelectrode. By contrast gastrocnemius moto-

neurones showed typical depolarizing synaptic potentials in response to single shocks to the gastrocnemius nerves. In some of these motoneurones, when these test shocks were preceded (approximately 7 msec) by a volley in hamstring afferents the depolarization was reduced by as much as 50% throughout its time course. However, the inhibitory hamstring volley alone produced no hyperpolarization of the postsynaptic membrane. Excitability of such cells measured by stimulation through the microelectrode was found to be unchanged following these inhibitory volleys. Antidromic invasion was neither helped nor hindered by such inhibitory volleys, as could be shown when safety factor for invasion was reduced by hyperpolarizing currents.

171. STATIC VOLUME-PRESSURE CHARACTERISTICS OF EXCISED LOBES OF DOGS' LUNGS. N.R. Frank (intr. by J. L. Whittenberger). Dept. of Physiology, Harvard School of Public Health, Boston, Mass.

The static volume-pressure characteristics of individual lobes of excised dog lungs were determined in 11 adult dogs. After ventilation with oxygen for 15 min, the lungs were allowed to collapse and become gas free while the chest was open; immediately afterward, hemorrhage was induced until the heart stopped. The right lung was separated by bronchial transection into 3 lobes (usually the "lower lobe" comprised 2 discrete lobes), the left into 2 lobes. Each lobe was inflated by an airway pressure of 30 cm H_2O. Following volume equilibrium, gas volumes were measured during deflation at pressures ranging between 30 and 0 cm H_2O and expressed in ml/gm of tissue. In 8 experiments, successful measurements were made on all 3 divisions of the right lung. At each airway pressure the middle lobe was most compliant while the lower lobe was least compliant ($P < 0.01$). For example, at an airway pressure of 5 cm H_2O, the average gas volumes for the upper, middle and lower lobes were 10.2 ± 2.5, 11.6 ± 2.4, and 7.9 ± 1.4 ml/gm of tissue. In 10 experiments on the left lung the upper lobe was more compliant than the lower lobe ($P < 0.01$), the average gas volumes being 11.2 ± 2.4 and 9.7 ± 2.3 ml/gm of tissue. These results were not influenced by the position of the animal during the surgical procedure, nor by the sequence in which the lobes were studied.

172. SIMULTANEOUS MEASUREMENT OF PLASMA VOLUME IN MAN WITH T-1824 AND AN IMPROVED I^{131}-ALBUMIN METHOD. J. J. Franks and F. Zizza (intr. by J. H. Holmes). Dept. of Medicine, Univ. of Colorado School of Medicine, Denver.

Recently Reeve and Franks (1956) found that I^{131}-labeled albumin is readily lost from solution by adsorption to glassware and showed that this can lead to errors in the measurement of plasma volume. A number of investigators have reported the results of simultaneous measurements of plasma volume in man with T-1824 and I^{131}-albumin. However, these measurements were made without full awareness of the errors that can arise from adsorption and with insufficient precautions to prevent all significant adsorption to glassware. We have therefore made 13 simultaneous measurements with T-1824 and an improved I^{131}-albumin method in 12 subjects. Significant loss of labeled protein to glassware was prevented by addition of homologous plasma to the I^{131}-albumin used for injection and preparation of standards, and by using physiological saline containing carrier protein (2 ml plasma/100 ml) as diluent for the standards. In this series the I^{131}-albumin values averaged +4.9% higher than the dye values. At the time of each measurement the proportion of loosely bound I^{131} was also determined by precipitating the labeled protein with trichloroacetic acid and measuring the radioactivity in the supernatant. In different samples it varied from 2-6% of the total. If correction is made for the loosely bound I^{131}, on the assumption that it is removed quickly from the circulation, the average difference is reduced to +2.0% (S.D. 3.0). These findings indicate that when the above precautions are taken, the I^{131}-albumin method gives measurements in close agreement with measurements by the dye method.

173. DEVELOPMENT OF RENAL HYPERTENSION IN ADRENALECTOMIZED RATS. Melvin J. Fregly. Dept. of Physiology, Univ. of Florida College of Medicine, Gainesville.

The adrenal glands of the rat do not appear to be necessary for the development of experimental renal hypertension by kidney encapsulation. Bilaterally adrenalectomized male and female rats became hypertensive when both kidneys were encapsulated with latex envelopes whether or not they were adrenalectomized 2 wk prior to, or at the time of, kidney encapsulation. The type of hypertension produced in adrenalectomized rats did not differ from that of rats with intact adrenals with regard to time course of development of the elevated blood pressure (6-8 wk), maximal elevation attained (190-220 mm Hg), polydipsia and hypertrophied hearts. Completeness of adrenalectomy was assessed by measurement of: a) growth rate, b) rate of fall of body temperature on exposure to cold air (5°C), c) voluntary intake of water and .15 M NaCl solution offered simultaneously and d) gross observation at autopsy. Growth rates of adrenalectomized-encapsulated (A-E) rats were similar to those of adrenalectomized rats and slower than those of normal or encapsulated rats. When choice was offered between water and .15 M NaCl solution, the volume of salt solution which A-E rats ingested 2-3 wk after encapsulation was 86% of their total fluid intake. At 9-10 wk after operation, the NaCl intake was 49% of their total fluid intake. In the case of adrenalectomized rats the NaCl intake increased from 79-82% in the same period. Hence, the encapsulation of both kidneys of adrenalectomized rats tended to abolish their salt appetite.

174. CONSIDERATIONS ABOUT ELECTROTONIC SPREAD DERIVED FROM COMPUTED EFFECTS OF BIOLOGICAL WAVEFORMS. W. H. Freygang, Jr. and H. Wiener.* Lab. of Neurophysiology, Natl. Insts. of Health, Bethesda, Md.

The neural structures carrying the electrotonic spread were assumed to be small (0.5-5.0 μ) smooth cylinders. Values of 1000 ohm cm^2 and 1 $\mu f/cm.^2$ were taken for membrane resistance and capacity. Forty ohm cm was chosen for the protoplasmic resistivity. Variations in source impedance were neglected and the length was considered as semi-infinite. If a unit impulse is applied at the end of such a cylinder, the voltage at a distance x from the end and at time t is given by Campbell and Foster as

$$\frac{y}{2t\sqrt{\pi t}} \exp\left(-\frac{y^2}{4t} - 2\beta t\right)$$

where $y = x\sqrt{RC}$ and $\beta = G/2C$. R is the protoplasmic resistance, C is the membrane capacity, and G is the membrane conductance per unit length. Intracellularly

recorded action potentials were divided into a series of impulses and the effect of each impulse was calculated. The final waveform was the sum of the impulses and it was calculated for several distances. The relation between distance and latency of the peak voltage for that distance is almost linear. Peak voltages decline exponentially with distance. For different voltages, the initial velocity rises rapidly as the voltage is reduced. Velocity falls roughly exponentially with distance; the length constant of this fall for a given cylinder is fairly constant for small voltages. Therefore, small variations in length and threshold can markedly influence the amplitude and temporal sequence of afferent impulses and may provide a mechanism for integrative actions.

175. TISSUE HEMATOCRITS OF NORMAL, NEMBUTALIZED, SPLENECTOMIZED AND NEMBUTALIZED-SPLENECTOMIZED MICE. Julius J. Friedman (intr. by K. Wasserman). Biophysics Lab., Tulane Univ., New Orleans, La.

Tissue hematocrits were determined by measuring tissue plasma volume with radioiodoalbumin and tissue red cell volume with radioiron-labeled red cells. The results, expressed as percentage, are tabulated below:

	Normal	Nembutal	Splenect.	Nembutal-Splenect.
Liver	38.9	29.0	34.7	33.8
Kidney	24.0	31.2	23.1	28.9
Spleen	51.3	51.9	-	-
Lung	47.9	38.4	44.7	39.7
Intestine	29.2	26.8	21.7	30.6
Skin	32.2	30.2	35.6	28.7
Muscle	41.9	34.2	38.3	31.4
Total blood	35.5	34.0	40.0	34.4
Venous blood	48.2	38.2	48.8	39.0

Nembutal produces a drop in liver and lung hematocrit by increasing the plasma volume. The increase in kidney hematocrit reflects a large rise in red cell volume accompanied by a smaller rise in plasma volume. Although spleen hematocrit remains unchanged, both plasma and red cell volume exhibit increases. Changes in other tissues are variable. Administration of Nembutal to splenectomized mice produces an increase in liver plasma and red cell volume without any change in hematocrit; kidney and intestine hematocrit rise due to a large increase in red cell volume and a smaller rise in plasma volume; lung and total body hematocrit fall due to an increase in plasma volume; and skin and muscle hematocrit fall due to a drop in red cell volume.

176. ACID SECRETION BY ISOLATED TOTAL STOMACH AND STOMACH POUCH. M. H. F. Friedman, Hubert A. Appert* and Henry Hoff.* Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.

Study of the factors determining acid secretion by the isolated stomach of the dog was continued. Two types of stomach preparations were employed: 1) the whole stomach in situ with its entire blood supply controlled from extracorporeal sources, and 2) a pouch from a region of the corpus, in which the circulation, restricted to the splenic artery and vein, was maintained by the animal's own heart. The limiting factors of temperature, arterial and venous pressures, pulse pressure and flow rate of the gastric circulation, as well as A-V differences in oxygen and carbon dioxide, were measured. Since acid secretion was provoked by means of drugs, it was found necessary to determine their influence on oxygen up-take per se. The basal oxygen uptake as well as the blood flow of the non-secreting stomach was found to be unaffected by histamine in the doses used. In experiments on the stomach pouch preparation histamine provoked acid secretions when given subcutaneously, while in experiments on the total stomach secretion of acid was not elicited by histamine introduced directly into the vascular circuit. As measured by venous return volumes, the volume flow early after histamine administration was not appreciably increased by the onset of secretion from the pouch. Blood flow measurements made late after histamine administration were complicated by side reactions. Secretion from the total stomach in response to drugs other than histamine occurred only if the flow rate of blood was increased to at least twice the basal rate.

177. MECHANISM OF TRITON-INDUCED HYPERCHOLESTEREMIA. Meyer Friedman and Sanford O. Byers. Harold Brunn Inst., Mount Zion Hosp., San Francisco, Calif.

The mechanism by which Triton WR-1339 induces hypercholesteremia in the rat has been investigated further. It was found that within minutes following the administration of 100 mg of the detergent, a rise of plasma triglyceride first occurs. About an hour later, the plasma cholesterol and phospholipid also begin to rise. This period of accumulation continued for 36 hr after which time, a period of decline extending over 36-48 hr occurred. The period of decline corresponds to the period in which the Triton of plasma is disappearing. During the period of accumulation, the excess cholesterol in plasma was observed to be relatively non-diffusible as judged by analyses of liver and hepatic lymph samples. However, during the period of decline, such excess cholesterol was observed to become diffusible. The degree of hypercholesteremia after Triton injection was found to be independent of the rate of hepatic synthesis of cholesterol but significantly increased by prior feeding of cholesterol-enriched diets. Indeed hypercholesteremia could be induced in the totally hepatectomized rat given Triton if, in addition, sufficient triglyceride and/or phosphatide were administered. It would appear that Triton induces hypercholesteremia by first interferring with the normal disposition of triglyceride. The accumulation of this lipid in plasma then effects the sequestration in the same medium of both phospholipid and cholesterol. Body sources other than the liver are adequate to supply this excess cholesterol to the plasma. This mechanism of cholesterol accumulation emphasizes again the causal role of a prior rise in neutral fat and/or phospholipid in the genesis of some types of hypercholesteremia.

178. FACTORS CONTRIBUTING TO THE DIMINISHED VENTILATORY RESPONSE TO CO_2 OF PATIENTS WITH OBSTRUCTIVE EMPHYSEMA. H. W. Fritts, Jr.,* A. P. Fishman and A. Cournand. Dept. of Medicine, Columbia Univ., New York City.

The diminished ventilatory response of patients with emphysema to carbon dioxide has been generally attributed to a loss of sensitivity of the respiratory center, although it has been suggested that a disturbance in the mechanics of breathing may play a significant role (Fishman et al. Am. J. Med. 19:533, 1955). In order to determine the relative importance

of these 2 factors, measurements of ventilation, arterial pCO_2, and the work done on the lungs were made while subjects breathed mixtures containing 0, 3, 4, and 5% CO_2. Using the change in pCO_2 as an index of the stimulus delivered to the respiratory center, the response of the center was judged by the increase in work which an increase in pCO_2 produced. The influence of a disturbance in the mechanics of breathing was assessed from the increase in ventilation realized per increase in work performed. In 4 normal subjects, the average increment in work was .3 kg-M/min mm Hg increase in pCO_2. In 4 patients with emphysema and CO_2 retention this increment was approximately the same. However, the increment in ventilation derived from a unit increase in work averaged 30 l/kg-M in the normal subjects, and only 4 l/kg-M in the patients. These results suggest that the retention of CO_2 does not invariably damage the respiratory center, since in these 4 patients the diminished ventilatory response can be explained by a disturbance in mechanics alone.

179. INEFFECTIVENESS OF CARNITINE AS A CHOLINE SUBSTITUTE IN THE PREVENTION OF FATTY LIVERS. Irving B. Fritz and Phil Dupont.* Dept. of Physiology, Univ. of Michigan, Ann Arbor, and the Dept. of Metabolic and Endocrine Research, Michael Reese Hosp., Chicago, Ill.

Carnitine addition results in an increased conversion of labeled palmitate to ketones and CO_2 by liver slices and homogenates from normal rats (Acta Physiol. Scand. 34:367, 1955). The percentage increase was augmented when liver preparations from malnourished rats maintained on a 5% protein, choline-deficient diet were incubated in the presence of carnitine (Fed. Proc. 15:68, 1956). However, liver homogenates prepared from rats maintained on a 20% protein, choline-deficient diet did not display any impairment in the rate of fatty acid oxidation, and carnitine addition to such homogenates resulted in a normal percentage increase in conversion rate. To see if these findings could be related to fat mobilization impairments, 3 groups of weanling male rats were maintained on the 20% protein choline-deficient diet with various dietary supplements. Group 1 received no addition, group 2 received choline and group 3 received an equimolar amount of carnitine. After all groups had been on their respective diets for 3 wk, the rats were killed, and the lipid content of livers and carcasses were determined. Values for the average percentage hepatic lipid were as follows: 16.30 ± 1.64 for group 1; 5.28 ± 0.44 for group 2; and 13.81 ± 0.93 for group 3. Since carnitine supplementation did not prevent fatty liver formation, it appears that the methyl groups of carnitine are not labile, and that free carnitine is not an intermediate in those metabolic reactions that choline undergoes in the process of altering fat metabolism in vivo.

180. EFFECT OF BREATHING O_2 ON O_2 CONSUMPTION DURING EXPOSURE TO COLD. G. Froese* and A. C. Burton. Dept. of Biophysics, Univ. of Western Ontario, London, Canada.

Previous experiments suggested that breathing O_2 decreased the O_2 consumption of humans during exposure to cold. The O_2 consumption was measured by breathing for 6 min in a closed spirometer system, filled alternately with air and oxygen. O_2 was allowed to enter the spirometer at the same rate as it was used up by the subject, thus keeping the O_2 concentration constant. The results of 4 measurements on each of 10 subjects, dressed and at normal room temperature (25°C) showed no significant difference between breathing air and O_2 ($P > 0.6$). During the next experiments the room temperature was about 10°C. Two determinations of the O_2 consumption were made while the subject was covered with blankets, and 4 more after the nude subject had been exposed to the room temperature for 20 min. The movements of the bed, which was suspended and free to move in the longitudinal direction, were recorded during shivering. An electromyograph record was also obtained. For analysis the results were grouped into successive pairs of observations for breathing air and O_2. The average decrease in O_2 consumption when breathing O_2 was 19.7 cc/min/sq m which was significantly different from zero ($P < 0.001$). The increase in O_2 consumption in response to exposure to cold was reduced by 1/3 when breathing O_2. A similar analysis of the electromyograph recording showed a significantly lower level for breathing O_2 ($P = 0.03$). No significant difference was obtained for the recording of the movements of the bed ($P = 0.6$). (Supported by the Defence Research Board of Canada.)

181. EFFECTS OF ALTERED EXPIRATORY PRESSURES OR RESISTANCES UPON ARTERIAL OXYGEN SATURATION DURING ARTIFICIAL RESPIRATION. M. Jack Frumin, Norman A. Bergman and Duncan A. Holaday (intr. by L. Shettles). Dept. of Anesthesiology, Columbia-Presbyterian Med. Ctr., New York City.

Healthy adult patients were ventilated during succinylcholine induced paralysis with an artificial respirator which servo controlled the inflating pressure to maintain a fixed end tidal CO_2 tension. The respirator delivered 13-20% O_2 in N_2O and cycled 15 times/min with inspiration occupying one third of the cycle. Arterial O_2 saturations were determined spectrophotometrically. Expiratory pressures were maintained at +5 mm Hg in 7 patients while the end tidal CO_2 tension was 40 mm Hg. Lowering the expiratory pressure to -5 mm Hg caused the O_2 saturation to fall an average of 5% (range 0-12%). In 8 other patients, a resistance 3 mm in diameter and 3 mm long was inserted into the expiratory port while the end tidal CO_2 tension was kept between 27-37 mm Hg. Removal of this resistance caused the O_2 saturation to fall an average of 3% (range - 2-8%). The possible role of increased phasic changes in alveolar composition as the alveolar volume may become smaller during expiration with concomitant desaturation of pulmonary capillary blood will be discussed. The role of phasic changes in blood flow will also be considered.

182. ENVIRONMENTALLY INDUCED CHANGES IN CEREBRAL OXYGEN AVAILABILITY. N. B. Furlong and M. J. Schwarz (intr. by J. W. Heim). Aero Med. Lab., Wright-Patterson AFB, Ohio.

Permanent implantation of platinum electrodes in brain tissue of the cat has enabled polarographic measurement of oxygen availability in a variety of environmental circumstances. The marked increase in oxygen availability produced by 5-15% carbon dioxide has been confirmed at low and high ambient oxygen pressures as well as normal pressure. The oxygen blocking effects of cyanide and carbon monoxide have been compared. The effects of chlorophyllin injections on oxygen and carbon dioxide physiology have been investigated at normal and subnormal total pressure. The relationship between the "relative oxygen tension" and "oxygen availability" has been approached by comparing polarographic readings to arterial and venous oxygen saturations.

183. TACHISTOSCOPIC PERCEPTION IN MONKEYS. Joaquin M. Fuster (intr. by Robert B. Livingston). Univ. of California, Los Angeles.

Rhesus monkeys were trained to recognize stereometric objects presented tachistoscopically. Keeping the intensity of light constant, the proportion of correct responses and the time of reaction were considered at different durations of exposure. Under these experimental conditions the effects of changes in the acoustic background and the injection of LSD were studied. The effect of electrical stimulation of the brain stem reticular formation was also investigated. This is a preliminary report on the results of such experiments obtained from 4 animals. A tone presented simultaneously with the short flash exposure of the stimulus objects impaired recognition, as indicated by fewer correct responses and longer reaction times. LSD at low dosages (5-10 gamma/kg) produced also impairment of visual acuity and a lengthening of the time of reaction. One animal, under stimulation of the reticular formation with a mild current (100 μA, 300 cps) through chronically implanted electrodes, showed significantly higher percentages of correct responses and shorter reaction times than were obtained in the absence of reticular stimulation. Changes in gross behavior were not detected.

184. ATROPINE DETOXICATION IN MICE AND RATS. John D. Gabourel* and Robert E. Gosselin. Dept. of Pharmacology and Toxicology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

After i.v. injection of atropine labeled with C^{14} in the alpha position of the tropic acid moiety, approximately 25% of an injected dose was excreted in mouse urine as atropine, over 50% as conjugates with glucuronic acid, and the remaining 20-25% as intermediate oxidation products (probably p-hydroxyatropine and o,p-dihydroxyatropine) and a tropine-modified atropine. Tropic acid was not present in urine until 6-8 hr after injection; it amounted only to 1% of an injected dose. Rats were shown to metabilize atropine in a manner similar to mice. Experiments with hepatectomized rats showed that at least one of the essential steps in glucuronide formation was carried out in the liver. The kidneys too were able to modify atropine to a small extent but did not form glucuronides.

185. HUMAN AMNIOTIC FLUID: A SOURCE OF CLOTTING AND FIBRINOLYTIC AGENTS. Leo Gagliardi* and Mario Stefanini. Joseph Stanton Research Labs., Saint Elizabeth's Hosp., and Dept. of Medicine, Tufts Univ. School of Medicine, Boston, Mass.

Hemorrhagic tendency, characterized by thrombocytopenia, hypoprothrombinemia, fibrinogenopenia and, at times, active fibrinolysis follows entrance of amniotic fluid (AF) into the circulation. Thus, AF probably contains factors promoting coagulation and inducing fibrinolysis. Ten samples of steril amniotic fluid were collected by puncture of the amniotic sac. Fluid was centrifuged at 2,500 rpm/10 min/4°C; supernatant, still containing cellular elements, was used in all experiments, either fresh or stored at -20°C until used. Standard technics were employed for determination of single clotting factors and activities. Results follow: a) AF accelerated clotting of platelet-rich or platelet-poor recalcified human plasma; b) by classical prothrombin consumption test using platelet-free native plasma as substrate, AF enhanced utilization of prothrombin during clotting in at least half of our experiments. AF also caused faster formation of thrombin in a thrombin generation system and substituted for either plasma or platelet reagents in thromboplastin generation test. c) AF showed weak tissue thromboplastin-like activity; contained traces of prothrombin, but no demonstrable activity of labile and stable factor and no fibrinogen; it also exhibited often antithrombic activity; d) AF contained profibrinolysin (by one test) and a proactivator of fibrinolysis, stable at 85°C; it also showed antifibrinolytic activity. Thus, AF contains many factors capable of accelerating intravascular coagulation and of activating fibrinolysis. Amniotic fluids, however, differ considerably in coagulative properties, while their fibrinolysis activating properties are fairly constant. Quantitative data, relation of coagulative properties to cellularity of AF, localization of factors in different fractions of AF and their relation to the clinical syndrome of AF infusion will be discussed.

186. MICROELECTRODE STUDY OF SUPERIOR OLIVARY RESPONSES TO SOUNDS. Robert Galambos, Johan Schwartzkopff* and Allen Rupert.* Dept. of Neurophysiology, Walter Reed Army Inst. of Research, Washington, D. C.

The superior olivary complex in cat has 5 major subdivisions of which the trapezoid and accessory nuclei and the S-shaped segment are most prominent. In barbitalized animals microelectrodes have been passed in 50-100 μ steps from the ventral side through these nuclear masses while sounds (clicks, tone, noise) were presented to the ears. Postmorten histological confirmation of all electrode tracks was obtained. Each ear evokes a characteristic slow wave response in all the subdivisions, the contralateral ear however being very poorly represented in the S-shaped segment. Single units have been isolated in all segments, some being equally well driven by each ear, others being driven by only one. Units in the S-shaped segment are aroused mainly by ipsilateral stimulation. The various units responded differently to tones, to diotic clicks presented in a simulated localization experiment, and to noise, many being activated by only some of these stimuli. When the responses observed are correlated with the anatomical location of the electrode tip at the time of recording, certain general statements about the different functions being performed in the separate subdivisions of the superior olive emerge.

187. EFFECTS OF AMINOPHYLLINE AND DIAMOX ON OXYGEN-INDUCED RESPIRATORY DEPRESSION IN PULMONARY EMPHYSEMA. Morton Galdston and Vincent W. Hollis, Jr.* New York Univ. Research Service, Goldwater Mem. Hosp., New York City.

Studies in 2 advanced emphysema patients indicate that intravenous aminophylline (6.5-11.5 mg/kg) alone and together with oral Diamox (3.4-4.5 mg/kg) every 12 hr is effective in treating oxygen-induced respiratory depression and CO_2 retention. Comparisons were made between minute ventilation in room air and the first 12 and last 4 min of 1 hr oxygen inhalation and between arterial pCO_2 and pH in room air and the last 4 min in oxygen. Twenty cc aminophyline (0.5 gm) or 0.85 N saline were administered intravenously during the 15th-20th min of oxygen. Means of 3 studies in control and Diamox periods are presented. Minute ventilation was depressed to the same degree in both patients during the first 12 min in oxygen in control and Diamox periods (-20 to -26%; -14 to -19%). Minute ventilation was unaffected by intravenous saline. Though it commonly returned to control levels with continued oxygen inhalation, periodic respiration

persisted and arterial pCO_2 rose 3-10 mm Hg and pH fell 0.02-0.06 U. Aminophylline promptly overcame respiratory depression during oxygen inhalation and sustained minute ventilation 15-25% above room air levels. Arterial pCO_2 fell 7-11 mm Hg (50-43; 74-63), pH rose 0.06 U (7.40-7.46; 7.32-7.38); when administered with Diamox, again arterial pCO_2 fell 11-13 mm Hg (50-39; 74-61), while pH remained unchanged (7.40) or fell (7.32-729) as compared with oxygen-saline levels.

188. SODIUM AND POTASSIUM EXCRETION AFTER INJECTION OF ALDOSTERONE INTO RENAL ARTERY. William F. Ganong, Patrick J. Mulrow and George W. Hollinger (intr. by L. L. Bennett). Dept. of Physiology, Univ. of California Med. School, Berkeley, and Dept. of Medicine, Stanford Univ. School of Medicine, San Francisco.

The rate of change of sodium and potassium excretion after aldosterone administration has been studied in the dog. Bilateral adrenalectomy and insertion of an indwelling cannula in the left renal artery (Barger et al. Fed. Proc. 15:8, 1956) were performed in 3 female dogs. Another dog was adrenalectomized and unilaterally nephrectomized. Seventeen to 20 days post-operatively, under light pentobarbital anesthesia, the abdomen was opened and the ureters catheterized. Multiple, serial measurements of the creatinine clearance and sodium and potassium excretion by each kidney were made before and after the injection of 5 or 10 gamma of aldosterone into the left renal artery. The unilaterally nephractomized dog received aldosterone intra-aortically. All animals showed decreased sodium and increased potassium excretion in both kidneys without consistent changes in GFR. The time of onset and magnitude of the changes were not significantly different in the injected and the uninjected kidney and were similar to those following intra-aortic injection. The fall in sodium excretion began in 5-30 min, reached a peak in 50-100 min and declined thereafter. The rise in potassium excretion generally but not invariably followed a similar time course. It is concluded that the injection of aldosterone into the renal artery has no grossly greater or more rapid effect on the injected kidney than on the opposite kidney. (Supported by Research Committee, Berkely Campus.)

189. PULMONARY BLOOD FLOW AND RESISTANCE CHANGES IN DOGS WITH ATRIAL SEPTAL DEFECTS AND AORTIC INSUFFICIENCY. Mary Gaspar, William G. Pogue and Edward Hawthorne (intr. by Evelyn M. Anderson). Dept. of Physiology, Howard Univ., Washington, D. C.

A comparative study of the pulmonary blood flow, pulmonary resistance, renal blood flow, sodium para-aminohippurate (PAH) space and related parameters was made in normal dogs and in dogs with either aortic insufficiency (AI) or atrial septal defects (ASD) alone, or combined ASD and AI. These parameters were determined by use of a constant infusion-slope technique following cardiac catheterization. In general, it was seen that dogs with ASD alone had a significantly lower pulmonary vascular resistance (average = 154 dynes sec cm^{-5}) than normal dogs (average = 267 dynes sec cm^{-5}). The pulmonary blood flow in ASD was approximately twice that of the normal value. In dogs with AI alone, these parameters did not differ from the normal dogs. Dogs with ASD and AI had a numerically normal pulmonary blood flow and slightly elevated pulmonary resistance. Data in support of these observations will be presented and their possible implications discussed.

190. TWENTY-FOUR HOUR RHYTHMICITY OF URINE VOLUME AND BLADDER EMPTYING IN HAMSTERS. Mona R. Gault (intr. by J. D. Thomas). Dept. of Physiology, State Univ. of Iowa, Iowa City.

Studies on 24-hour rhythm of locomotor activity of hamsters, recorded by tambour technique, indicate that this species is strongly nocturnal. In a 12-hr cycle of light and darkness, about 90% of the daily major activity occurred in the darkness, and about 88% during the same solar period when kept in continuous darkness. Similar results were found with bladder emptying even when the animals were kept in continuous darkness. Simultaneous records were made of activity, time of voiding, volume of urine and water consumption. Four animals were studied in 6 experimental combinations of light, darkness and cold. In over 6 months of almost daily recordings, the animals were found to react as a homogenous group (mean control voidings/animal/day 5.1, 6.9, 5.1, 6.9; mean percentage of voiding per animal for 12-hr periods of darkness 58.4, 56.2, 57.0, 55.7). Only the conditions of cold altered, by dilution, the rhythmicity displayed by the activity and bladder emptying. Although the animals remained nocturnal as far as activity and bladder emptying were concerned, during the conditions of cold with control conditions of light and darkness or cold with continuous darkness, significantly more locomotor activity and bladder emptyings occurred during the solar day than formerly. Number of voidings increased with the animals in cold. It appeared that in cold, the animal could not afford to maintain the rigid rhythmicity displayed under other conditions. (Research aided by Society of Sigma Xi.)

191. CHEMICAL CHANGES IN STRUCTURAL COMPONENTS OF BRAIN CORTEX DURING STIMULATION. Alexander Geiger, Ishiro Yamasaki and Liana Nebel (intr. by F. A. Gibbs). Neuro-Psychiatric Inst., Div. of Psychiatry, Univ. of Illinois, Chicago.

It has been shown previously, that nitrogenous compounds are released from nucleic acids and lipids of the sensory-motor cortex, when afferent nerves were stimulated. Further experiments demonstrated the following chemical changes in the brain cortex: during stimulation of the brachial plexus of cats at 75 cps for 30 sec, the adenine- and cytidine content of the nucleic acids increased, the uridine content remained at pre-stimulation level and the guanidine content varied. Increased amounts of adenine and of guanine were found in the nucleic acids after Metrazol convulsions. The galacto-lipid content decreased during and significantly increased after Metrazol convulsions. During stimulation a loss of nitrogen occurs from substances extracted by hot perchloric acid from the cortex. Compounds other than nucleic acids are also involved. (Supported by the Natl. Inst. of Neurological Diseases and Blindness, PHS.)

192. EFFECT OF LYSERGIC ACID DIETHYLAMIDE (LSD-25) AND SEROTONIN ON ADULT CORTICAL BRAIN CELLS IN TISSUE CULTURE. R. S. Geiger (intr. by Percival Bailey). Div. of Psychiatry, Univ. of Illinois, Chicago.

Effects of LSD-25 in concentrations of 0.0002-0.001 μ/ml on the structure of neurons are described. Within 15-20 min of exposure granules are seen to move away from the nuclear membrane and are dispersing into small uniform aggregates throughout the cytoplasm except in the axoplasm. Staining with thionine shows uniform dispersal of Nissl substance

in small aggregates. Production and extrusion of nucleoproteins from the nucleolus into the cytoplasm is accelerated, and changes of optical density are seen in the nucleoplasm and areas of the nucleolus. A decrease in osmophlia is seen in the cytoplasm and some cytoplasmic granules, while the nucleus and nucleolus become increasingly osmophilic in some areas. Contraction of the whole neuron and motility changes in synaptic areas are seen. Prolonged exposure to LSD-25 causes cytoplasmic vacuolization in neurons accompanied by dissolution of Nissl substance. The motility of glia is inhibited by LSD-25. Serotonin in concentrations of 0.5-2 μg/ml induced repetitive pumping movements of the neurons hitherto not seen under other conditions. A change in the pattern of movements in the synaptic areas occurred which was different than that caused by LSD-25. Prolonged effects of serotonin on neuronal and glial structure and motility are also demonstrated. (Supported by the Dept. of Public Welfare, Illinois.)

193. RIGIDITY OF SPINAL ORIGIN. Samuel Gelfan, A. A. Eurnekian* and I. M. Tarlov.* Dept. of Neurology and Neurosurgery, New York Med. College, New York City.

In the permanent rigidity of dog's hind legs resulting from temporary ischemia of the lumbosacral spinal cord, neuronal structures in the cord which normally exert a regulatory influence are absent, rendering the surviving motoneurons "spastic." The responses of these motoneurons to all afferent impulses so far tested, which do not synapse directly upon them, are either reduced or absent, whereas the monosynaptic response is more easily elicitable than in normal dogs. The normal inhibitory influence of muscle tension and contraction upon the monosynaptic response also is either reduced or absent. The lengthening reaction characteristic of decerebrate rigidity cannot be demonstrated in these rigid dogs. Reaction to painful stimuli to hind legs also is minimal or absent. The rigidity is of spinal origin and is not significantly altered after mid-thoracic spinal cord transection. This rigidity is not purely an exaggerated stretch reflex phenomenon since alpha and gamma motoneuron activity (as well as the hypertonus in the chronic preparation) is not completely abolished by deafferentation (bilateral S_2 - L_3 rhizotomy). In this respect, the inhibitory role of the anoxically destroyed neurons is comparable to that of the anterior cerebellum in the deafferented decerebrate preparation. These spinal interneurons must include at least some with which supra-spinal, as well as peripheral, impulses normally synapse, before reaching motoneurons.

194. EFFECTS OF TEMPERATURE ON ELECTRICAL ACTIVITY OF THE BULLFROG SPINAL CORD. John A. Gergen* and Edgar L. Gasteiger. Dept. of Physiology, Harvard Med. School, Boston, Mass.

Effects of temperature (4-20°C) on electrical properties of the isolated bullfrog spinal cord have been studied in over 20 preparations. All cords were initially cooled to 4-6°C for dissection. At lower temperatures (4-8°C) propagated activity in the VR becomes increasingly synchronized and may appear as a single peak of several millivolts amplitude approximately 16-20 msec after DR stimulation. This activity is usually followed by a prolonged period (up to 150-200 msec) of depressed excitability as measured by test stimulation of the same or adjacent DR. This depression develops over a period of 20-30 msec and parallels the VR slow potential. Upon raising the temperature, this period of depression is altered to a period of hyperexcitability with a like time course of development and duration. A similar change is produced by reducing the conditioning volley. A later less prominent hyperexcitability lasting 1-5 sec is seen at all temperatures; this is replaced by depression during low frequency repetitive stimulation (1-2/sec). Antidromic VR stimulation is ineffective in producing similar conditioning. Higher temperatures (8-20°C) are accompanied by prolongation of VR discharge with increasing amplitude and duration of the VR slow potential. These effects will be discussed in terms of the action of low temperature on synaptic events and on the motor neuron excitability. (Supported by the Kirkland fellowship of the Natl. Paraplegia Fndn.)

195. EFFECTS OF EXOGENOUS INSULIN UPON URINARY NONPROTEIN NITROGEN IN CORTISONE-TREATED RATS. Phillip E. Gertler (intr. by D. J. Ingle). Ben May Lab. for Cancer Research and Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

Cortisone causes a rise in urinary NPN when given in excess to normal rats over a period of several days. Large doses cause an accompanying glycosuria (steroid diabetes). Insulin corrects the negative nitrogen balance of depancreatized diabetic rats. The effect of insulin upon the nitrogen balance of rats with steroid diabetes was studied in the present experiments. In normal (300 gm) fasted rats "glucagon-free" insulin in excess of 0.1 U/24 hr increases urinary NPN as does cortisone in excess of 1 mg/24 hr/rat. Insulin can aggravate the NPN loss in fasting rats treated with cortisone. In normal force-fed rats regular insulin in excess of 4 U/24 hr/stimulates NPN loss. Cortisone in daily doses above 3 mg increases NPN loss and causes glycosuria; in lower doses there is a rise in urinary NPN without glycosuria. Cortisone effect on NPN loss is greater in the fed than in the fasted rat. High doses of insulin may suppress the glycosuria of the rat with steroid diabetes without suppression of NPN loss. Cortisone-induced loss of weight is not corrected by insulin. Rats having mild glycosuria from partial pancreatectomy show a rise in urinary glucose and NPN when treated with cortisone; simultaneous treatment with insulin suppresses the glycosuria but not urinary NPN. It is concluded that insulin does not suppress the "catabolic" response to cortisone. (Supported by a grant from the Eli Lilly Co.)

196. FACTORS INFLUENCING COURSE OF MERCURIAL DIURESIS IN PATIENTS WITH MILD CONGESTIVE HEART FAILURE. Donald Gibbons,* Jacob Grossman and Raymond E. Weston. Montefiore Hosp., New York City.

The administration of a mercurial diuretic during intravenous infusion of vasopressin (Pitressin) (1-3 mμ/kg/hr) in normal human subjects results in an essentially osmotic diuresis superimposed upon the initial hypertonic flow. This increment of flow induced by the diuretic (tubular rejectate), hypertonic at first, approaches isotonicity as the urine flow increases suggesting that a small amount of water unaccompanied by solute is reabsorbed. With increasing flow the distal isosmotic reabsorptive capacity is promptly exceeded so that, despite a small rise in free water reabsorption, the urine becomes more dilute and the rejectate nearly isosmotic. This hypothesis was tested by performing similar studies in patients with congestive heart failure whose reduced GFR and normal tubular function should result in a greater proportional distal reabsorption of water before the

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exceeded capacity is reflected by a rise in urine flow. Renal hemodynamics, electrolyte, water and total osmolal excretion were studied in patients with mild failure who, at rest, could tolerate sufficient salt to provide an adequately hypertonic urine. At the onset of diuresis the negative free water clearance and consequently the tubular rejectate concentration were significantly greater than those encountered in normal subjects. The small difference in negative free water clearance (1-2.5 ml/min) in the presence of a 20-30% reduction in GFR is compatible with the generally accepted concepts governing partition of proximal and distal reabsorption. Separate studies of maximal water excretion following intravenous water loading in these patients further supported these concepts.

197. BIOLOGICAL EFFECTS OF HIGH OXYGEN TENSIONS (MOTION PICTURE). D. L. Gilbert,* J. Frost,* D. Caccamise* and R. Gerschman. Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Paramecium caudatum were photographed through the window of an especially designed pressure chamber. During the control period in air, they show their normal slipper shape and rapid motion. After about 20 min in 20 atmospheres of oxygen, the onset of the following progressive changes can be seen: decreased forward movement, accentuated rotational motion and rounding of the body. Marked decrease in activity permits contractile vacuoles to become observable. Analagous effects can be seen in Tetrahymena pyriformis similarly exposed to 20 atm. of oxygen. They become almost spherical. A pronounced darkening at the anterior end appears as a condensation of protoplasm. A longer time is necessary for the appearance of these changes at lower oxygen pressures. These unicellular organisms clearly show that oxygen tension can change cellular structure. Oxygen toxicity in mammals is illustrated with 20-gm female mice exposed to 6 atm. of oxygen. Violent tonic and clonic convulsions reflecting serious C.N.S. derangement appear in about 10 min. Disability of voluntary movements can also be observed. After about 20 min of exposure to 6 atm. of oxygen, the mice enter the exhaustion stage. Dyspnea and gasping show profound involvement of the respiratory system. Death occurs in approximately 45 min. This film illustrates the toxic effects of oxygen on 3 experimental organisms, but it should be emphasized that high oxygen tensions are toxic to all living matter. The effects depend specifically upon the oxygen tension and not upon the physical factor of total pressure alone.

198. PAPER ELECTROPHORETIC STUDIES OF SERUM PROTEINS IN EXPERIMENTAL SHOCK INDUCED BY MASSIVE SOFT TISSUE WOUNDS. R. W. Gillette, A. R. Mansberger, Jr., J. H. Oppenheimer, B. F. Caldwell and E. G. Neff (intr. by C. M. Herget). Biophysics Div., Directorate of Med. Research, Chem. Warfare Labs., Army Chem. Ctr., Md.

Electrophoretic separation of serum proteins was performed on goats which had sustained lethal soft tissue wounds. Two groups of animals were used: 1) a control group and 2) a group receiving antibiotic (penicillin or aureomycin) treatment. The control group had a mean survival time of approximately 18 hr, while the antibiotic-treated group survived an average of approximately 50 hr. There was an early increase in the relative percentage of the alpha-2 globulin fraction in both groups. After 24 hr of survival, the beta globulin fraction of the antibiotic-treated animals increased markedly. Unwounded animals under similar antibiotic regimens did not show serum protein changes. An additional small group of animals received lethal doses of gram negative endotoxin. There were no changes in the serum proteins in these animals.

199. EFFECT OF PENTOBARBITAL SODIUM UPON SOME CIRCULATORY PARAMETERS. J. P. Gilmore (intr. by Stanley W. Handford). Dept. of Physiology, Naval Med. Field Research Lab., Camp Lejeune, N. C.

In spite of the extensive experimental use of pentobarbital sodium as an anesthetic there is still some question as to the effect of this drug upon the various circulatory parameters of the dog. During the course of a current study experiments were completed in which the effect of pentobarbital sodium upon the cardiac output, plasma volume and arterial pressure of the normal dog was determined. Measurements were made prior to anesthesia and for 4 hr following the injection of the drug in an amount equal to 30 mg/kg. The results of these experiments indicated that cardiac output and plasma volume did not change significantly during the time intervals studied. Mean arterial pressure demonstrated no change until late after the administration of the anesthetic. The well-known tachycardia, hemodilution, rectal temperature decrement and less well-known leukopenia, which follow the injection of pentobarbital sodium, are also described.

200. COMPARATIVE STUDY OF SERUM AND GASTRIC MUCOPROTEINS. George B. Jerzy Glass, Marilyn Rich* and Loukia Stephanson.* Gastroenterology Research Lab., New York Med. College, New York City.

In order to determine relationships between muco-proteins from human serum and gastric juice following materials were prepared and studied: 1) serum orosomucoid (Winzler); 2) serum mucoprotein (Weimer 3), 4) gastric glandular mucoprotein and mucoproteose (Glass - Boyd). After dialysis and lyophilization these materials and non-fractionated serum and gastric juice were submitted to paper-electrophoresis (Spinco apparatus, Whatman #1 paper, borate buffer, pH 9.0, L/2 0.12, 5-1/2 hr, 0.4 ma/cm), stained with amido-black 10 B, Köiw's PAS stain and FS light green, and scanned in Analytrol. Each material was studied for: protein content (biuret and Folin-Ciocalteu), total hexoses (Badin's modification of Shetlar's tryptophane method and Lustig's orcinol technic), hexosamine (Rimington), fucose (Dische), uronic acid (Dische's carbazol and Fishman's naphto-resorcinol methods), and sialic acid (Bial reaction, Ayala diphenylamine technic, and Seibert tryptophane - perchloric acid method). Anodic mobility of orosomucoid equals that of peak M_2 of gastric juice (Glass. Glin. Chem. 2:269, 1956), is slightly faster than that of serum mucoprotein and gastric mucoproteose (peaks M_3 and M_4 of gastric juice), and slower than that of glandular mucoprotein (peak M_1 of gastric juice) and pepsin. Orosomucoid contains slightly more protein, hexosamine and fucose and about double amount of sialic acid and hexoses than serum mucoprotetin. Both exhibit no intrinsic factor activity when assayed by hepatic uptake of $Co^{60}B_{12}$ on pernicious anemia patients. The content of tyrosine (8.2%), hexosamine (5.0%) and hexoses (12.5-16.1%) in orosomucoid is intermediary between that of gastric glandular mucoprotein (11.2%; 2.9%; 4.8-6.0% resp.) and gastric mucoproteose (5.0%; 28.4%; 29.0-35.3% resp.). The content of sialic acid (11.6-14.6%) is 7-10 times greater, and that of fucose (1.2%) 2-7 times lower in orosomucoid than in gastric

mucous substances. Orosomucoid and serum mucoprotein are chemically and electrophoretically different from glandular mucoprotein and mucoproteose of gastric juice. (Supported by Grant-in-Aid A-68 (C4) from the Natl. Insts. of Health, PHS, and Irwin Strasburger Mem. Med. Fndn.)

201. LOWER BRAIN STEM FACILITATION OF CARDIOVASCULAR ACTIVITY. Richard L. Glasser (intr. by William R. Amberson). Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore.

In cats with vagi cut and common carotids tied, low midpontile decerebration effected by occlusion of the basilar artery produces an enduring marked increase in blood pressure and heart rate in addition to the classical manifestations of apneusis and decerebrate rigidity. Blood pressure elevations of 40-100 mm Hg and heart rate increases of 50-120 beats/min are generally obtained. In animals with vagi intact low midpontile decerebration results in a transient fall in heart rate and blood pressure followed by a return to normal heart rate and blood pressure or, more often, by a moderate tachycardia and hypertension. Subsequent vagotomy induces the striking increases in heart rate and blood pressure described above. Apneusis and a considerable augmentation of existing decerebrate rigidity accompany these rises. Surgical transection of the brain stem rostral to the basilar artery occlusion has no further effect on either the cardiovascular, respiratory or somatic hyperactivity. Examination of the brain stem sections reveals that the triple hyperfacilitated responses are obtained with only the medulla and caudal third of the pons functioning. In some experiments subsequent progressive destruction of the caudal pons and rostral medulla abolishes augmented activity in the following sequence: cardiovascular hyperactivity first, apneusis second, decerebrate rigidity last. Current theory associates decerebrate rigidity and apneusis with facilitatory mechanisms in the reticular formation of the brain stem. Presumably, augmentation of cardiovascular activity here reported is also due to reticular facilitation.

202. INFLUENCE OF GOITROGENS ON INDUCTION OF DECIDUOMA IN RAT UTERUS. Stanley R. Glasser (intr. by T. R. Noonan). Dept. of Radiation Biology, Univ. of Rochester School of Medicine, Rochester, N. Y.

Induction of deciduoma in the rat uterus produces an increase from 57.5 mg/100 gm body weight in the control horn to 211.9 in the traumitized horn. This increase of 268.5% is accompanied by a rise in uterine water content from 77.3-85.1%, a decrease in protein concentration (on a wet basis) of 12.5-8.45% and in β-glucuronidase activity from 638.9 units/gm/hr to 370.6 and an increase in the percentage of glycogen from 21.5-29.5. Four-week pretreatment with goitrogenic drugs, mixed with chow diet in such concentrations as to be of approximately equal antithyroid activity, alters these parameters. Deciduoma analyzed after feeding 0.1% 1,2 mercaptoimidazole produce the following values. Weight increase: 185.2%; % H_2O: 74.9-82.5; % protein: 12.9-8.50; β-glucuronidase: 322.9-252.0 and % glycogen from 17.5-23.7; 0.5% thiouracil allows a 181.2% weight increase, % H_2O: 76.7-82.8; % protein: 11.3-10.0; β-glucuronidase: 580.4-424.8 and glycogen from 22.7-21.7; 0.1% propylthiouracil allows an increase in weight of 157.7%; % H_2O: 77.5-83.1; % protein: 12.9-8.10; β-glucuronidase: 498.8-319.6 and % glycogen: 17.9-22.6; 0.1% 6-methylthiouracil allows a 280.7% increase in weight, % H_2O: 77.5-83.7; % protein: 11.8-10.7; β-glucuronidase: 391.7-199.4 and an increase in the percentage of glycogen from 17.6-21.7.

203. MONOAMINE OXIDASE INHIBITION (IN SITU SEROTONIN), AND CEREBRAL SYNAPTIC INHIBITION. Melvyn I. Gluckman,* Victor H. Cohn, Jr.,* E. Ross Hart* and Amedeo S. Marrazzi. VA Research Labs. in Neuropsychiatry, VA Hosp., Pittsburgh, Pa.

Cerebral synaptic inhibition by intracarotidly injected serotonin was demonstrated by us in the cat. In order to further evaluate the significance of this finding, it was necessary to determine the action of serotonin in situ. A readily practicable procedure that can be expected to elevate cerebral serotonin is inhibition of cerebral monoamine oxidase. The results of such inhibition, induced by intracarotidly injected iproniazid, were therefore studied in: 1) rats, for detailed determination of enzymatic changes, and 2) cats, where correlation with electrical recording of transmission in cortical synapses transcallosally activated, as previously described, was carried out. Rat brain homogenates separated into 3 fractions (mitochondria, soluble and debris) showed major monoamine oxidase (MOA) activity only in the mitochondria and debris, the soluble portion being almost devoid of activity. The brain MOA activity essentially showed no difference between rats either stunned or anesthetized with sodium pentobarbital. With intracarotid injection of iproniazid (as a MOA inhibitor) a differential inhibition of the MOA activity, greater in the homogenate of the injected side, was demonstrated when the hemispheres were individually removed a short time (30-60 sec) following the injection. In cats a significant differential inhibition of MOA, and presumed differential accumulation of in situ serotonin, was observed to correlate well with the recording of cortical synaptic inhibition on the side with the greater enzyme inhibition. The data therefore indicate that the cerebral synaptic inhibitory action of serotonin is exercised equally well by endogenous supplies accumulated in situ probably at the cortical synapses as by injected serotonin.

204. AUDITORY DISCRIMINATION AFTER ABLATION OF TEMPORAL AND INSULAR CORTEX IN CAT. Jay M. Goldberg,* Irving T. Diamond* and William D. Neff. Lab. of Physiological Psychology, Univ. of Chicago, Chicago, Ill.

In earlier experiments (Neff, Fisher, Diamond and Yela. J. Neurophysiol. 1956; 19:500-512; Butler, Diamond, and Neff, J. Neurophysiol., in press; Diamond and Neff, in press) it has been found that retrograde degeneration of the caudal portion of the medial geniculate body is related to ablation of the temporal and insular cortex which lies between the ventral boundary of the auditory projection areas II and Ep and the rhinal fissure. Behavioral tests of frequency and pattern discrimination have now been made after bilateral ablation of this ventral region; auditory areas AI, AII, and Ep were spared. After this ablation, simple frequency discrimination can be learned at about the same rate as in the normal intact animal. A profound loss occurs in ability to learn to discriminate between groups of tones which differ only in temporal patterning (high, low, high vs. low, high, low). From the known cortical and subcortical connections of this region of temporal and insular cortex, no ready hypothesis can be suggested to account for the severity of the deficit in discriminatory ability. Operated animals appear to behave normally in their home cages and respond appropriately to shock in testing

apparatus. (Aided by a grant from the Office of Naval Research.)

205. INFLUENCE OF "TIME FACTOR" IN RADIATION EFFECTS ON BIOLOGICAL SYSTEMS. Anna Goldfeder and Grace E. Clarke.* Cancer Research Lab., Dept. of Hosps. and of Biology, Grad. School of Arts and Sciences, New York Univ., New York City.

Experiments were undertaken in this laboratory to evaluate the significance of the time-dose-relationship of x-ray effects on biological subjects. Several observations along these lines were reported (Fed. Proc. 15:77, 1956). This study deals with evaluation of effects produced by a specific dose of x-rays applied in one and in several equal exposures at various time-intervals. Two tumor types were employed: DBAG spindle-cell, faster growing, and $DBAH_1$, slower growing, both indigenous to DBA-strain mice. In one series of experiments, a dose of $10{,}000_r$ was applied to both types of tumors in 1, 2, 4, 8 sessions at 24-hr intervals. The greatest effect was exerted on the DBAG-tumor when $10{,}000_r$ were applied in 2 sessions and the least effect when applied in 8 sessions. Furthermore, in the latter scheme of treatments, significantly more distant metastases to various organs appeared among the treated mice. Observations made on the $DBAH_1$ tumor differ to some extent from those made on the DBAG tumor, and these will be presented. Different doses and other time-schemes of x-ray treatments have been investigated and observations are being accumulated. The role played by the inherent biological properties of the tumors in this type of experimentation will be brought forth. (Supported by a grant from the Natl. Cancer Inst., PHS.)

206. EFFECT OF RESERPINE ON STRESS RESPONSE OF ADRENALECTOMIZED ANIMALS. Maurice S. Goldstein and Estelle R. Ramey. Dept. of Physiology, Univ. of Chicago, Chicago, Ill., and Dept. of Physiology, Georgetown Univ. School of Medicine, Washington, D. C.

Previous work has shown that the use of peripheral autonomic blocking agents, such as banthine and dibenzyline, protects the normal and the adrenalectomized animal against some of the consequences of exposure to severe stress. In the experiments to be reported, the drug reserpine, which is believed to act on the central nervous system, was used. Male Sprague Dawley rats weighing 150-200 gm were traumatized by tumbling in the Noble-Collip drum; 90% of normal rats survived up to 350 revolutions of the drum at a speed of 40 revolutions/min. Fifteen percent of the adrenalectomized rats survived the same procedure. Pretreatment of both normal and adrenalectomized rats with 1 mg/kg of reserpine 24 hr before exposure to tumbling in the drum increased the ability of normal and adrenalectomized animals to survive this stress; 58% of the reserpine treated adrenalectomized animals survived up to 350 revolutions. Normal animals were enabled to tolerate previously lethal amounts of tumbling. These results are interpreted to mean that reserpine like the peripheral autonomic blocking agents diminished the intensity of peripheral tissue stimulation resulting from an applied stress, and thus protected the animal against the toxic effects of excessive neurhumoral release.

207. QUANTITATIVE INTERRELATIONSHIPS BETWEEN THROMPOPLASTIN, PROCONVERTIN-CONVERTIN AND CALCIUM IN PROTHROMBIN CONVERSION TO THROMBIN. Robert Goldstein, Gabriel Izak and Estelle Zonderman (intr. by A. S. Freedberg). Beth Israel Hosp. and Harvard Univ., Boston, Mass.

Thrombin formation was studied in a bovine system containing variable concentrations of lung thromboplastin, highly purified prothrombin (6,000-12,000 U/ml), purified preconvertin-convertin (100-3,000%), $BaSO_4$ adsorbed serum (2-200% in terms of serum Acglobulin activity) and calcium (0-0.027 M). When all are relatively concentrated, thrombin yield is 50% of the orthodox 2-stage prothrombin assay. This can be gradually increased to 100% if calcium is progressively reduced, reaching optimum between 0.0027-0.00135 M, below which thrombin formation is again impaired. Complete calcium deprivation prevents thrombin elaboration. At 0.027 M calcium, progressive dilution of thromboplastin results in rapid fall in thrombin evolution, reversible by bringing calcium to optimal range. With proconvertin-free prothrombin little thrombin evolves under any circumstance. The addition of varying concentrations of convertin complex profoundly influences velocity and amount of prothrombin conversion. The extent of thrombin evolution depends upon calcium and thromboplastin concentration in relation to convertin. At high convertin level, dilution of thromboplastin results in sharp fall in thrombin. Under the same conditions, hyperoptimal calcium concentration blocks prothrombin conversion entirely. This can be rectified by greatly augmenting both thromboplastin and convertin complex. The data suggest that with these reactants thrombin formation requires an ultimate thromboplastic agent, which arises from activation of the tissue moiety generally termed tissue thromboplastin by the convertin complex in the presence of calcium. The quantitative interrelationship of these entities is of utmost importance, under certain circumstances producing activating effects, while under others showing inhibitory properties. (Supported by the Office of the Surgeon General (DA-45-007-MD-171) and PHS (H-656).)

208. ANOXIC TOLERANCE OF HEART DURING PERFUSION AT VARIOUS TEMPERATURES. Frank Gollan and Innes A. Nelson.* Research Lab. and Surgical Services, Thayer VA Hosp., and Dept. of Surgery, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Selective hypothermia was produced in dogs by circulating, oxygenating and refrigerating venous blood in a small, plastic pump-oxygenator. The bypassed, contracting, hypothermic heart could be safely deprived of coronary flow for 15 min at 28°C and for at least 25 min at 21°C. During such periods of ischemia the electrocardiogram revealed severe, but reversible injury patterns. The myocardial oxygen tension fell gradually and rose steeply on release of the aorta. After restoration of oxygen tension, of rate and of force of contraction the myocardium could be subjected to succeeding periods of ischemia. The recording of oxygen tension with a motion sensitive platinum electrode showed the greatest amplitude of contraction at 22°C. During cardiac arrest induced by intracoronary injection of potassium citrate the safe period of myocardial ischemia could be prolonged to 1 hr at 20°C.

209. TWO NEW ENTITIES IN HUMAN THROMBOPLASTIN GENERATION OF FUNDAMENTAL AND CLINICAL SIGNIFICANCE. S. Gollub, Alex Ulin and Joyce Black (intr. by Floyd J. Wiercinski). Hahnemann Med. College and Sidney Hillman Med. Ctr., Philadelphia, Pa.

The Thromboplastin Generation Test was investigated with the enzyme thromboplastinase. Two new entities were found to be generated by mixture of rupture platelets, alumina plasma and serum. Calcium is not required for their elaboration. One of these entities is destroyed by thromboplastinase, is non-dialyzable and is converted in the presence of calcium to a necessary reactant for the subsequent formation of the powerful accelerator characteristic of the Thromboplastin Generation Test. Its properties are similar to "thromboplastic lipid." It has been named Lipid Analogue or "L.A." It seems to be the material in blood analogous to tissue thromboplastin. The other of these entities is not destroyed by thromboplastinase, is dialyzable and is needed as a co-reactant to the functioning of the powerful accelerator characteristic of the test. We have named it Dialyzable Component #1 or "D.C.-I." It is suggested that plasma thromboplastin is unlike classical tissue extra thromboplastin on the basis of non-lability to thromboplastinase, lability of activity and requirement of the new entity, "D.C.-I." The stability, properties and preparation of LA and DC1 are discussed. Activity curves and methods of assay are presented. The reduction of these factors in hemophilia A and hemophilia B as well as by Dicumarol administration is demonstrated. A working hypothesis of the generation of human blood thromboplastin is presented.

210. BIOSYNTHESIS OF ADRENALINE AND NORADRENALINE BY SYMPATHETIC NERVES AND GANGLIA. McC. Goodall and Norman Kirshner.* Dept. of Physiology, Duke Univ. School of Medicine, Durham, N. C.

Minced bovine and canine sympathetic nerves, sympathetic ganglia and parasympathetic nerves were incubated with C-14 labeled tyrosine and with C-14 labeled dihydroxyphenylalanine (dopa). Incubations were carried out in Kreb's phosphate buffer (Biochem. et Biophys. Acta 4:249, 1950) containing glucose and pyridoxal phosphate. Subsequent separation of adrenaline, noradrenaline and hydroxytyramine from the incubated mixtures was by column chromatography (Kirshner and Goodall. J. Biol. Chem., in press). Bovine and canine sympathetic nerves and ganglia incubated with either labeled tyrosine or labeled dopa formed radioactive hydroxytyramine and noradrenaline; the amount of radioactive adrenaline formed was slight. Vagi nerves incubated with labeled dopa yielded significant amounts of radioactive hydroxytyramine; when incubated with labeled tyrosine the radioactivity in the hydroxytyramine was insignificant. The amounts of radioactivity in the adrenaline and in the noradrenaline fractions formed from vagi nerves incubated with labeled dopa or with labeled tyrosine did not exceed that of the boiled controls. Evidence herein shown lends support to the concept that noradrenaline is the neurohormone of the sympathetic nerves. (Supported by Natl. Inst. of Health Grant H-2546.)

211. IN VITRO EFFECTS OF SULFONYLUREAS ON ISOLATED FROG MUSCLE. D. R. H. Gourley and R. H. Dodd.* Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.

In isolated frog muscle insulin accelerates the oxidation of lactate (Am. J. Physiol. 179:378, 1954) and the influx of potassium ions (Canad. J. Biochem. & Physiol. 34:893, 1956). Although they also cause hypoglycemia in intact animals, carbutamide and tolbutamide do not exert the same effects as insulin in isolated frog muscle but each influences the metabolism differently. Carbutamide, like sulfanilamide, inhibits the oxygen consumption of muscle in vitro and potassium is lost from the tissue. Tolbutamide greatly increases the oxygen consumption of this tissue and there is a slight increase in potassium influx. The effect of tolbutamide on oxygen consumption was inhibited by sodium azide which suggested that the increased utilization of oxygen was due to lactate oxidation. Although no contractions were observed visually and the muscles retained their irritability throughout the experiments, tolbutamide caused a great decrease in muscle glycogen and an accumulation of lactate. The small effect on potassium influx is probably a secondary effect due to lactate oxidation. Thus a change in the sulfonylurea molecule from a $-NH_2$ group (carbutamide) to a $-CH_3$ group (tolbutamide) completely reverses the effects of the compound on frog muscle metabolism.

212. RESPONSE OF YOUNG AND OLD MICE TO COLD. B. Grad, V. A. Kral (intr. by R. A. Cleghorn). Gerontologic Unit, Allan Mem. Inst., McGill Univ., Montreal, Canada.

Old female C57BL mice (16-22 months) showed a significantly higher mortality rate on exposure to cold than mature young females (4-9 months). The largest difference in mortality was observed at 6°-7°C, although very highly significant differences were also observed at 9-11°C. Temperatures which killed 100% of unadapted old mice within 24 hr killed none of the old mice within this limit when they were taken to this temperature gradually, an indication that old mice can adapt to the cold. Two-thirds of adapted old mice died after a week at a temperature which failed to kill any adapted young mice. Therefore, adaptation to cold is less effective in the old. Exposure to a "cold room" temperature of 9-11°C resulted in a significantly greater increase in the oxygen consumption, food intake and blood sugar in the young than in the old; body weight decreased significantly less in the young. Blood lymphocyte counts decreased reliably more in the young than in the old and the erythrocyte count increased significantly more in the young. Significant differences due to age in the eosinopenic, neutrophilic or liver glycogenolytic response to cold could not be detected. (Supported by funds given to the Allan Mem. Inst. of Psychiatry by the Federal-Provincial Mental Health Grants.)

213. BASAL METABOLIC RATE IN MAN IN SEMI-STARVATION AND REFEEDING. Francisco Grande, Joseph T. Anderson, Henry L. Taylor and Ancel Keys (intr. by Carlos Martinez). Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.

Basal heat production (BMR), total body water, extracellular fluid space and nitrogen balance were measured in normal young men during control, semi-starvation and recovery. A normal diet was given during control and recovery. In semistarvation, the diet consisted of carbohydrate plus a daily supplement of 4.5 gm NaCl and vitamins. Group I (13 men) received daily 3280, 1010 and 5300 Calories during these periods and group II (12 men) 3966, 1000 and 5384 Calories. Physical activity, including treadmill walking costing about 1200 Calories daily during control, was constant throughout. "Active cell mass" was computed as gross weight less fat, extracellular fluid and bone mineral. The gross BMR decreased in all the subjects during semistarvation, the average decline being 17.1% after 19 days (group I) and 21.4% after 13 days (group II). The BMR was also decreased when computed per unit of gross weight, per unit of body surface, per unit of gross body weight to the 0.73

power and per unit of "active cell mass" (or of total body nitrogen). The average percentage decline being, respectively 8.7, 13.4, 9.8 and 11.9 for group I and 14.2, 18.4, 15.9 and 16.2 for group II. All p values for the differences are < 0.001. After 1 wk of refeeding the gross BMR returned precisely to the control value in each group and remained at that level until the end of the observations. The mechanism of these BMR changes will be discussed.

214. REACTIONS OF GASTRIC TISSUES TO NON-CARCINOGENIC IRRITANTS. Rhoda Grant. Dept. of Clin. Science, Univ. of Illinois, Chicago.

Thymol, thread and "norite" (carbon) particles were embedded in cauterized or uncauterized areas of submucosa along the greater curvature of the glandular stomach of rats. In other cases a thread was drawn through the gastric wall from lumen to serosa and tied in place. The histological changes in serial sections were studied at intervals from 50-403 days after the operation; and compared with the changes seen in previous experiments in which carcinogenic hydrocarbons were embedded. From 45-100 days in 5 of 7 rats a path through which foreign matter (except carbon) was being ejected extended through the mucosa. In this path the normal pattern was replaced by a cystic mucosa in which parietal and chief cells were replaced by hyperplastic less specialized cells. Encapsulated thread and muscle formed the base of the lesion. In 2 rats, mucosa and muscularis mucosae were normal above the encapsulated thread. From 101-200 days, in 2 of 6 rats, ejection paths for foreign matter through abnormal mucosa were present. In 4 cases the mucosa was normal above the encapsulated thread. From 201-403 days (6 rats) the mucosa and muscularis mucosae were normal above the encapsulated thread in all cases. Carbon particles were present in stroma, in nuclei and cytoplasm of glandular cells from base of glands to crypts, and caused no cytological or structural changes in the mucosa. In contrast to the reaction to carcinogenic hydrocarbons, no tumors were produced in these experiments, and epithelial downgrowth was limited by muscle and fibrous tissue which surrounded the thread.

215. INFLUENCE OF SUCROSE AND CASEIN ON PLASMA CHOLESTEROL IN ANIMALS ON PURIFIED DIETS. W. C. Grant and M. J. Fahrenbach.* Research Div., American Cyanamid Co., Pearl River, N. Y.

Day-old White Leghorn male chicks were placed on various purified diets for 4 wk, following which the plasma cholesterol values were measured. Diet I contained glucose, 64%; soy bean protein (Drackett), 25; methionine, 0.7; while diet II contained sucrose 60%; crude casein, 20; gelatin 8; cystine 0.4. Both diets contained calcium gluconate, 5%; bone ash, 2; salt mixture, 2 plus a complete vitamin mixture. After 4 wk on these diets without added cholesterol, the average plasma cholesterol values were for chicks on diet I, 177 mg/100 cc and for diet II, 184 mg. Chicks fed the same diets with 3 gm/cholesterol and 3 gm corn oil added/100 gm of diet had plasma cholesterol values of 334 mg (diet I) and 606 mg (diet II). Plasma lipoprotein values were higher in chicks fed diet II plus cholesterol than in chicks fed diet I plus cholesterol. In further experiments it was found that when the protein source was held constant and only the carbohydrate vaired, again the animals consuming the diet containing sucrose plus cholesterol had higher cholesterol values than did those on glucose plus cholesterol. These results suggest that the uptake of dietary cholesterol is greater in chicks on purified diets containing sucrose as the sole carbohydrate than is the case in chicks on similar diets with glucose as the carbohydrate.

216. EFFECT OF PROTEIN AND THIOURACIL ON THE ADULT MALE RAT FOLLOWING DIETHYLSTILBESTROL ADMINISTRATION. Cyrus M. Greenberg (intr. by Marion A. Reid). Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.

Adult male rats fed 18% casein received 0.1 mg of diethylstilbestrol subcutaneously for 20 days. These animals were then recovered for an additional 20 days while receiving 0.5% thiouracil. Pair-fed controls were run concurrently. During recovery, rats receiving stilbestrol throughout the initial phase of the experiment always were in higher nitrogen balance than the non-injected animals. Thiouracil depressed nitrogen balance. Thiouracil-treated rats not previously given estrogen, while initially returning to positive nitrogen levels, eventually returned to negative nitrogen values. Although stilbestrol had no influence on liver water or fat content, animals receiving thiouracil during recovery had significantly less water and fat concentration than their pair-fed controls. The protein concentration of the liver was significantly higher in non-treated pair-fed rats than in animals receiving stilbestrol and/or thiouracil. On the other hand, thiouracil increased the relative protein of the liver above that of control values either in the presence or absence of prior estrogen treatment. Feeding thiouracil during recovery, following stilbestrol treatment, did not influence testes protein concentration, although thiouracil had a suggestive depressing effect in normal rats. Furthermore, thiouracil following estrogen administration hindered the recovery of total testes protein while having no effect when fed to non-estrogen treated rats. Diethylstilbestrol caused a significant decrease in the total lipid content of the testes and thiouracil administered throughout recovery did not alter this effect.

217. SITE OF INTESTINAL CONVERSION OF CAROTENE TO VITAMIN A. Ruven Greenberg. Univ. of Illinois Med. School, Chicago.

After a carotene meal, in vivo or in vitro, the autofluorescence of carotene and vitamin A are easily visualized histochemically in unstained frozen tissue sections of rat small intestine. In vitro, the first recognizable vitamin A is seen after 5-15 min. It is first visualized within the intestinal lumen just adjacent to the tips of the villi. Subsequently the vitamin A is recognized within the intestinal mucosa, in the lining epithelial cells, in cells in the lamina propria and in the lacteals. No carotene is visualized histochemically within the mucosa, even though small amounts of unchanged carotene are known to be absorbed. The data suggest that the conversion of carotene to vitamin A occurs as an extracellular process.

218. SPATIAL SUMMATION OF PAIN. L. C. Green and James Daniel Hardy (intr. by A. M. Stoll). Aviation Med. Acceleration Lab., U. S. Naval Air Development Ctr., Johnsville, Pa., and Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia.

Spatial summation of pain was investigated by Hardy et al. in 1940, and they concluded from this study that there is no spatial summation for pain in the sense that the pain threshold for a large stimulated area was no lower than that for a small area. However

Breig, in 1953, supported the idea that noxious impulses are spatially summated by showing that with linear scratch stimulation the pricking pain threshold is reached only after a certain length of the stimulation scratch. For the present study cutaneous pain thresholds were determined on the forehead over areas from 2.47 cm^2 by recording skin temperature during exposure to thermal radiation. From these measurements thresholds for the various size apertures were determined. The values obtained for the pain threshold regardless of the size aperture used, fall between 43.5° and 46.8°C. The pain threshold using the 16 cm^2 area is 44.6°C ± 0.69, while for the 2.47 cm^2 aperture the pain threshold is 45.7°C ± 0.71. These values are significantly different (p - 0.01) indicating a minimal degree of spatial summation or possibly a shift in sensory end point due to increased heat sensation associated with the large apertures.

219. INHIBITORY EFFECT OF PANCREATIC SECRETIN ON GASTRIC SECRETION. Herbert B. Greenlee,* Enrique H. Longhi,* Jose Delgadillo Guerrero,* Thomas S. Nelsen* and Lester R. Dragstedt. Dept. of Surgery, Univ. of Chicago, Chicago, Ill.

Pancreatic Secretin (Lilly) was found to exert a marked inhibitory effect on the secretion of gastric juice. In dogs with Heidenhain pouches the secretion of gastric juice induced by feeding was almost completely inhibited by the repeated intravenous injection of pancreatic Secretin. On the other hand, pancreatic Secretin had no effect on the gastric secretion induced in these animals by histamine. Pancreatic secretin caused a marked inhibition of the gastric secretion induced by insulin hypoglycemia in dogs with Pavlov pouches or vagus innervated total stomach pouches. On the other hand, pancreatic secretion had little or no inhibitory effect on the gastric secretion induced by feeding the dogs with vagus denervated total stomach pouches. In animals prepared with both a Heidenhain pouch and a total pancreatic fistula the intravenous injection of Secretin caused a simultaneous stimulation of pancreatic secretion and an inhibition of gastric secretion induced by feeding. It is suggested that pancreatic Secretin inhibits the nervous and gastric phases of gastric secretion but has little effect on the intestinal phase of gastric secretion.

220. DISTRIBUTION OF A RADIOMERCURY LABELED DIURETIC (CHLORMERODRIN) IN TISSUES OF MARINE VERTEBRATES. Roger L. Greif and Marilyn R. DuVigneaud.* Marine Biological Lab., Woods Hole, Mass., and Dept. of Physiology, Cornell Univ. Med. College, New York City.

Radiomercury, administered as labeled chlormerodrin, is found in high concentration in renal tissue of mammals and certain poikilotherms, including a fresh water teleost. (Giebisch and Dorman, personal communication). In the present work the distribution of radiomercury was examined in the tissues of several marine species. The flounder (Paralichthys dentatus) nephron has both glomerulus and proximal segment, the toadfish (Opsanus tau) nephron has a proximal segment but no glomerulus, and the dogfish (Mustelus canis) nephron has glomerular, proximal tubular and distal tubular elements. The ratio of mercury concentration in the kidney to that in plasma in these species (K/P ratio) was always greater than 2, and under some conditions exceeded 500. Where lymphoid tissue was present in the kidney (flounder and toadfish) the mercury concentration was directly related to the proportion of microscopically visible renal structures. High concentrations of mercury were also found irregularly in liver, bile and spleen, but not in the gills, stomach, heart or gonads. A high U/P ratio for mercury in the toadfish indicates a secretory mechanism, as noted in dogs (Borghgraef, Kessler and Pitts. J. Clin. Invest. 35:1055, 1956). The relation of these findings to mercurial diuresis will be discussed.

221. OVARIAN LACTIC ACID IN NORMAL AND HYPOTHYROID RATS FOLLOWING GONADOTROPHIN ADMINISTRATION. J. R. Greslin* and J. H. Leathem. Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.

The ovarian weight increase following chorionic gonadotrophin administration is greater in hypothyroid than in euthyroid rats. To appraise ovarian biochemical changes related to this augmentation, lactic acid was estimated in normal and hypothyroid rats with and without gonadotrophin. Thirty-day-old rats were fed 0.5% thiouracil in a 20% casein diet. Following a 10-day prefeeding period, 10 IU of chorionic gonadotrophin were injected subcutaneously daily for 20 days; animals were also autopsied after shorter treatment periods. Twenty injections of chorionic gonadotrophin increased ovarian weight from 19.6-110.1 mg in euthyroid rats and from 18.4-375.9 mg in hypothyroid rats. Ovarian lactic acid was increased from 62.9-112 μg by the gonadotrophin in euthyroid rats and from 79-157.7 μg in hypothyroid rats. These data reveal that hypothyroidism alone favors a modest increase in lactic acid and that response to gonadotrophin is associated with a marked increase in ovarian lactic acid. Preliminary assessment was made of uterine, adrenal and thyroid lactic acid in normal and hypothyroid states.

222. BULBAR CONTROL OF RESPIRATORY MUSCLES. E. M. Grim,* B. A. Lawing* and L. M. N. Bach. Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.

Studies were made on the complex of intercostal, abdominal and diaphragmatic muscles involved in inspiratory and expiratory movements induced by electrical stimulation of the bulbar respiratory areas using stereotaxically oriented bipolar electrodes in cats decerebrated by midcollicular section. It was shown that normal values of tidal exchange require either intact phrenic nerves or at least 60% of the intercostals. Neither tidal expiration or any other aspect of tidal respiration requires the abdominal muscles. Tidal inspiration is independent of the intercostals between T6 and T12 and is irregularly affected by cord section at T12 or at T9. However tidal ventilation is markedly reduced following cord section at T9 and is almost eliminated by cord section at T6. Artificially induced IRV is unaffected by section of either phrenic nerve but is reduced by 50% following section of both phrenics. Root section between T9 and T6 also causes a reduction of induced IRV by 50% when phrenics and remaining intercostals are intact. A pronounced additional reduction occurs when cord section at T9 follows root section between T9 and T6 implying that cord elements other than cells supplying the intercostal nerves are involved. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis.)

223. PANCREATIC SECRETION IN THE RAT. Morton I. Grossman. VA Ctr. and Univ. of California Med. Ctr., Los Angeles.

Chronic pancreatic fistulas were prepared in rats by ligation of the common bile duct above the pancreas and introduction of a polyethylene cannula into

the intramural portion of the common bile duct at its entry into the duodenum. In 20 rats weighing between 300 and 420 gm the mean basal rate of secretion was 0.42 ml/hr and the mean concentration of protein in the juice was 2.4%. Rate of secretion and protein concentration did not diminish over a 4-day period of observation. Rate of secretion and protein concentration were unaltered by introduction into the duodenum at the rate of 1 ml/hr of the following solutions: 0.9% NaCl, 0.1 N HCl, 6% casein hydrolysate, and 5% sodium oleate. Intravenous injection of secretin produced moderate stimulation of volume rate which was detectable at doses of 4 units/rat and maximal at 10 units/rat.

224. DEFIBRILLATORY EFFECTS OF LOW SODIUM SOLUTIONS ON VENTRICULAR FIBRILLATION IN THE RABBIT'S ISOLATED HEART. L. Grumbach (intr. by A. M. Lands). Pharmacology Section, Sterling-Winthrop Research Inst., Rensselaer, N. Y.

The ability of a perfusion medium with lowered sodium content to defibrillate ventricles in fibrillation induced by $CaCl_2$ injections was studied electrocardiographically in isolated rabbit hearts perfused in the Langendorff apparatus. Solutions, in which approximately half of the normal amount of sodium chloride was replaced by osmotically equivalent amounts of sucrose, defibrillated without interfering with the ability of the ventricles to conduct normal impulses arriving from the auricles. Reversion to a sinus rhythm occurred without a pause. The action of these solutions closely resembles that of normal solutions containing excess KCl or procaine. These experiments support the hypothesis that the defibrillatory effect of the latter agents is due to their ability to interfere with the entrance of sodium ions in the rising phase of the action potential. This mechanism is understandable if ventricular fibrillation involves the discharge of premature impulses having a low safety factor of propagation from one or more ectopic foci. The demonstration of the defibrillatory effect of low sodium solutions is complicated by a second effect of these solutions. This resembles the electrocardiographic effects of a normal solution to which calcium has been added. In normal hearts, low sodium solutions can reproduce the specific effect of extra calcium in prolonging the P-R interval and shortening the Q-T interval. However, there remains an important difference between the 2 solutions: the QRS duration is prolonged by low sodium but shortened by high calcium. Low sodium solutions could also imitate the ability of high calcium solutions to initiate ventricular fibrillation, particularly when the potassium level was also lowered.

225. CONTINUOUS CARDIAC OUTPUT RECORDER UTILIZING THE FICK PRINCIPLE. Arthur C. Guyton, Charles A. Farish* and Ray J. Nichols.* Dept. of Physiology and Biophysics, Univ. of Mississippi School of Medicine, Jackson.

A recorder that automatically measures the A-V oxygen difference measures the rate of oxygen utilization, and then computes the cardiac output has been constructed. A photoelectric system measures the optical densities of arterial and venous blood at a wave length of 640 mμ. This system then subtracts the difference between the two optical densities, and the electrical output has been shown to be a linear function of arteriovenous oxygen difference. The apparatus measuring the rate of oxygen utilization employs a paramagnetic oxygen meter. The expired air from the animal passes through a system of flow regulators so that the output from the oxygen meter is a direct measure of the rate of oxygen utilization. Then, by means of an analogue computer the electrical output from the A-V oxygen difference analyzer is divided into the electrical output from the oxygen utilization analyzer, giving a continuous record of cardiac output. (Supported by a grant-in-aid from the American and Mississippi Heart Assocs.)

226. CARDIAC EFFECTS OF NOREPINEPHRINE AND METHOXAMINE FOLLOWING ACUTE CORONARY EMBOLIZATION. S. V. Guzman, J. W. West, A. T. Faulk and S. Bellet (intr. by E. L. Foltz). Div. of Cardiology, Philadelphia Gen. Hosp., and Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia.

In both intact and open-chest dog preparations anesthetized with pentobarbital sodium, the anterior descending or circumflex branch of the left coronary artery was catheterized via one carotid artery and embolization by 0.1 cc doses of 1% lycopodium spore suspension was done until a steady hypotensive state was reached during which arterial blood pressure was reduced by 50% or more, and left atrial pressure was elevated. Pressures were recorded in both atria, pulmonary artery, left ventricle and femoral artery. In the open-chest preparation, myocardial contractility was measured by a Walton strain gauge. Norepinephrine and Methoxamine were injected before and after coronary arterial embolization into the following sites: a) coronary artery, b) femoral ven and c) descending aorta. Coronary arterial injection of norepinephrine (0.02 - 0.04 mcg/kg) resulted in an immediate increase in myocardial contractility, a rise in femoral arterial blood pressure of approximately 40 mm Hg and a fall in left atrial pressure of approximately 5 mm Hg; whereas, methoxamine (4.0-8.0 μg/kg i.a.) produced none of these effects. However, doses greater than 10 μg/kg i.a. decreased myocardial contractility while left atrial pressure either increased or remained unchanged. Intravenous injection of norepinephrine (1 μg/kg) increased arterial blood pressure and decreased left atrial pressure. Methoxamine (100 μg/kg i.v.) also raised systemic arterial blood pressure but elevated left atrial pressure. Norepinephrine (1 - 5 μg/kg) injected into the descending aorta resulted in an immediate increase of systemic and left atrial pressures, followed by a delayed further rise in arterial blood pressure and a fall in left atrial pressure. Methoxamine (100 μg/kg) similarly injected resulted only in an immediate elevation of systemic and left atrial pressures.

227. ESTIMATION OF ARTERIAL CO_2 TENSION BY REBREATHING TECHNIQUE. Jack D. Hackney, Charles H. Sears and Clarence R. Collier (intr. by J. E. Thomas). Dept. of Internal Medicine, College of Med. Evangelists, Los Angeles County Hosp. and White Memorial Hosp., Los Angeles, Calif.

The method of continuous infrared alveolar CO_2 analysis has been shown to estimate the arterial or effective alveolar CO_2 tension with reasonable accuracy in poliomyelitic patients and normal subjects. When the method is used on subjects with abnormalities of distribution of blood and gas in the lung and with abnormalities of intrapulmonary mixing and emptying, the end-tidal CO_2 tension no longer reflects the arterial CO_2 tension. However, it was shown that the mixed venous CO_2 tension could be determined by the infrared rebreathing technique. Theoretical analysis by Suskind and Rahn (J. Appl. Physiol. 7:59, 1954) indicates that the arterio-venous CO_2 tension

difference is reasonably constant regardless of the cardiac output. Therefore it should be possible to estimate the arterial CO_2 tension once the mixed venous tension is known. This possibility was investigated in a group of 60 consecutive patients with heart or lung disease. Many patients had marked emphysema with CO_2 retention. The end-tidal and the mixed venous CO_2 tension was determined. A constant arterio-venous CO_2 tension difference of 6 mm Hg was assumed in all cases and the arterial PCO_2 calculated. The mean error of the infrared method as compared with the measured arterial CO_2 tension was + 0.2 mm Hg with a standard deviation of 2.9 mm Hg (maximum errors observed - 5.3 and +5.3).

228. EFFECT OF pH CHANGE UPON SYSTEMIC LARGE AND SMALL VESSEL RESISTANCE. Francis J. Haddy, Malcolm Fleishman* and Jerry B. Scott.* Army Med. Research Lab., Ft. Knox, Ky.

The effect of pH change upon total and segmental foreleg vascular resistance has been studied in 14 pentobarbital anesthetized dogs. Pressures were measured in the brachial artery, foot pad small artery (0.5 mm), subcutaneous small vein (0.5 mm) and cephalic vein of the nerve intact, nerve blocked and nerve blocked plus regitinized foreleg. Total extremity blood flow rate was maintained constant by interposing a pump in the brachial artery. Blood pH was varied from 7.6-7.0 by hyperventilation and ventilation with 20% CO_2 over a 15-min period. The changes in total resistance in the nerve intact, nerve blocked, and nerve blocked regitinized foreleg were -0.07±0.25, -0.15±0.12, -0.22 0.13 mm Hg/ml/min respectively. The arterial and small vessel resistance changes were +0.25±0.23, +0.10±0.17, +0.05±0.10 and -0.34±0.13, -0.21±0.17, -0.26±0.13 respectively. Venous resistance failed to change significantly in any of the three preparations. These data appear to demonstrate that an increase in hydrogen ion concentration results in active small vessel dilatation through a direct effect upon vascular smooth muscle. This small vessel dilatation does not decrease total resistance in the intact leg because of directionally opposite active changes in artery resistance. The latter response appears to be related to central nervous connections and perhaps to circulating or locally released epinephrine, norepinephrine or unknown vasoactive substances.

229. ARTERIAL HEMODYNAMICS IN AORTOILIAC STENOSIS IN MAN. Henry Haimovici and Doris J. W. Escher.* Surgical and Med. Divs., Montefiore Hosp., New York City.

This investigation is based on a correlative study of clinical, arteriographic and intra-arterial pressure findings in patients with aortoiliac syndrome. The intra-arterial pressures were recorded simultaneously from one brachial and both femoral arteries through Statham strain-gauge manometers connected to 18-gauge Cournand needles. The simultaneous records included the systolic, diastolic and mean pressures, as well as the ECG. The most striking hemodynamic alteration associated with aortoiliac stenosis was reflected in changes of the pulse pressure (PP), especially in its femoral to brachial ratio (PP F/B). Using this ratio as a criterion, the hemodynamic changes were classified as indicating minimal, moderate or marked stenosis. A segmental arterial occlusion with a well-developed collateral circulation may exhibit hemodynamic changes similar to those of a very marked stenosis. An aortogram may be helpful in differentiating the 2 conditions. The degree of stenosis induced by the thrombus was always more pronounced than the clinical and angiographic findings would indicate. In a previous work (Haimovici. Fed. Proc. 13:64, 1954; Haimovici. Surgery 36:1075, 1954) it was shown that up to about 60% of arterial diameter reduction no significant hemodynamic changes occurred and that a critical point existed at about 70% reduction in diameter. In the present investigation, it was found that 82% reduction in cross-section area of the lumen was necessary before significant manifestations of blood flow impairment took place. The PP F/B ratio appears to be an index of the degree of stenosis. Its physiological and clinical significance are stressed.

230. A SYSTEM DETECTED BY BIOASSAY IN PLASMAS OF PATIENTS WITH ESSENTIAL HYPERTENSION. Stephen Hajdu and Edward Leonard (intr. by Robert Akers). Labs. of Kidney and Electrolyte Metabolism and Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

As reported previously (J.C.I. 35:708, 1956), 1.0 ml of plasma from patients with severe essential hypertension typically causes disappearance of the staircase phenomenon of the frog heart. Contracture generally follows. One ml of normal plasma has little or no effect on staircase. The following experiments show that activity is caused by more than one component. Serum of a hypertensive becomes inactive after dialysis against a large volume of water. After exposure of the heart to dialyzed hypertensive serum, addition of 0.5 ml of normal serum (itself inactive) causes activity. Thus a dialyzable factor (component S), present in all sera, is needed for activity. The non-dialyzable factor (component L), remaining in dialyzed serum, is capable of binding strongly to the frog heart. Component L also occurs in normal serum. It can be demonstrated by successive applications of normal serum. Although the first ml has little effect on staircase, each following addition is associated with increased activity, since from each ml of serum an additional amount of component L is bound to the heart. With each washing between additions the activity disappears, the increasing effect being due to cumulative binding of component L. Components L and S also occur in beef serum. Beef component L can react with human component S and vice versa. It appears that patients with essential hypertension exhibit increased activity of a system present in all humans and other species also.

231. URINARY EXCRETION OF RATS EXPOSED TO COMBINATIONS OF ADVERSE ENVIRONMENTAL FACTORS. Henry B. Hale, Roy B. Mefferd, Jr. and Herman H. Martens (intr. by P. C. Tang). School of Aviation Medicine, USAF, Randolph AFB, Texas, and Biochemical Inst., Univ. of Texas, Austin.

Exposure of rats for 12 wk to low barometric pressure, to cold, or to heat resulted in distinctly different urinary excretion patterns. Where low pressure induced a blanket reduction in the 24-hr fasting excretion of certain electrolytes and nitrogenous compounds, cold had much the opposite effect, and heat a somewhat intermediate influence. The marked dissimilarities observed suggest specific environmental effects. To test this possibility, a factorial design was utilized and groups of rats numbering 30 each were exposed for 12 wk to low barometric pressure at neutral temperature or to low barometric pressure in combination with cold or heat. Control groups were exposed for similar times to different thermal conditions only. Individual 24-hr fasting urine collections made every

other week were analyzed for 5 electrolytes and 13 nitrogenous constituents. Although not all of the urinary constituents reflected the effects of the dual factors, additive effects were observed in some cases. The effects of heat or cold on certain constituents were definitely influenced by the added factor of low pressure, but the effect was not always additive.

232. MANIFESTATION OF SKIN INCOMPATIBILITY IN PARABIOTIC RATS. C. E. Hall and O. Hall.* Depts. of Physiology, Med. Faculty, Stockholm, Sweden, and Univ. of Texas Med. Branch, Galveston.

It is known (Billingham et al. Nature 178:514, 1956) that skin heterotransplants between genetically dissimilar mice provoke an immune reaction. Initial grafts may survive about 10 days, but later grafts between the same donor and recipient survive only half as long. We have noted that in some pairs of parabiotic rats the skin anastomosis begins to regress both anteriorly and posteriorly 2-4 wk after union until there remains only a short pedicle overlying the abdominal muscle anastomosis which finally amputates. To determine whether this was due to an immune response, pairs which showed evidence of regression were separated after 25 days in union and reunited 10 days later. The same was done with pairs showing no evidence of regression. Both groups behaved identically for the first 10 days, but then the skins of the animals in the first group separated along the entire length of the anastomosis between the 10th and 14th day. Animals of the second group showed normal healing. Neither group showed other pathologic changes. It is concluded that transplantation immunity affecting skin is manifest in parabiotic rats by a gradual and progressive skin breakdown at the anastomosis and that this immune reaction is not the cause of any of the other systemic changes thought to be due to immunity which occur spontaneously in parabiotic rats.

233. SHIVERING ONLY VARIABLE SOURCE OF HEAT IN INTACT UNACCLIMATIZED DOGS UPON EXPOSURE TO COLD. H. T. Hammel and James Daniel Hardy (intr. by Margaret McCouch). Dept. of Physiology, Univ. of Pennsylvania Med. School, Philadelphia.

Direct and indirect calorimetric studies have been made on 3 intact unacclimatized dogs while exposed to temperatures between 8° and 18°C for 5 hr. Continuous recording of the oxygen uptake was obtained with the Beckman Oxygen Analyzer Model F-2 in an open-circuit system. The response time of the system was rapid; 90% of the correct analysis was registered within 1 min of a change in oxygen uptake. Twenty-two examples have been observed in 10 5-hr runs during which time the dog would cease shivering and its metabolic rate (oxygen consumption) would return to the resting level, that is the resting metabolic rate obtained in a neutral environment. The duration of these periods at the resting level ranged from 1-15 min and 2-5 min was the average duration. These periods of cessation of shivering were found to occur at any time during the run. When shivering stopped the metabolic rate returned to the resting level; therefore it appears that the only additional heat produced by the unacclimatized dog in response to a cold environment is produced by muscular activity. (Supported in part by Contract DA-49-007-MD (496) with Army Med. Research Lab., Fort Knox, Ky.)

234. FAILURE OF DIBENZYLINE TO BLOCK SOME PERIPHERAL EFFECTS OF THYROXINE. G. E. Hammond,* G. A. Gronert* and N. B. Schwartz. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Some peripheral effects of thyroxine may be mediated by adrenergic substances. Since thyroxine alters neuromuscular function (Fed. Proc. 15:166, 1956) it was decided to determine if Dibenzyline alters this effect. Four groups of rats were treated for 2 wk as follows: drinking water containing .05% propylthiouracil; thyroidectomy; 50 μg d,l-thyroxine daily; untreated. To half of each group, 2 mg/kg Dibenzyline was injected, twice on the 14th day and once on the 15th day when the animals received 35 mg/100 gm barbital and the resting O_2 consumption determined. Then the gastrocnemius was stimulated indirectly at various frequencies. Hypothyroid rats exerted more tension and hyperthyroid rats less tension than controls. No difference was noted in resting oxygen consumption or muscle function between Dibenzyline and non-Dibenzyline treated animals. To determine the more chronic effects of Dibenzyline, 4 groups of animals received .05% PTU in their drinking water. Daily injections of a blank solution, or 2.5, 10, or 50 μg d,l-thyroxine were given. One half of each injection group also received 2 mg/kg Dibenzyline daily. After 2 wk of treatment the animals were anesthetized with 6.5 mg/100 gm pentobarbital and the oxygen consumption and thyroid weight determined. There were no differences found between Dibenzyline and non-Dibenzyline treated animals. It is concluded, that Dibenzyline does not alter the effect of chronic thyroxine treatment on oxygen consumption, muscle function or thyroid weight. (Supported in part by a research grant, B-768, from the Natl. Inst. of Neurological Diseases and Blindness, PHS.)

235. EFFECTS OF LOW O_2 AND HIGH CO_2 CONCENTRATIONS IN INSPIRED AIR ON LOCAL CEREBRAL CIRCULATION. Douglas B. Hansen, Mary R. Sultzer, Walter H. Freygang and Louis Sokoloff (intr. by Seymour S. Kety). Natl. Inst. of Mental Health, Bethesda, Md.

Alteration in the arterial blood tensions of O_2 and CO_2 are known to produce marked changes in cerebral circulation. However, the degree of uniformity of these responses in the various component structures of the brain have not yet been quantitatively determined. To determine the distribution of the local responses, the previous described technique (Fed. Proc. 14:85, 1955) for the determination of local cerebral blood flow in the cat by means of the radioactive inert gas, CF_3I^{131}, was applied in unanesthetized animals. The necessary catheterization of the femoral artery and vein was performed under Vinethene anesthesia. Following a 1-hr period of recovery from anesthesia, the cats were permitted to breathe the experimental gas mixture introduced at a rate of 15 l./min into a leucite box fitted over their heads. A 15-min equilibration period was allowed before the experimental procedure was performed. Similar experiments were done on control animals breathing room air. The results show a moderate increase in all cerebral tissues produced by the inhalation of 5% CO_2 and a marked increase produced by 10% CO_2. The effects of 10% O_2 were intermediate. Although the increase in blood flow produced by these inspired gas mixtures was distributed in both gray and white matter of the cerebral cortex, sub-cortical structures and the spinal cord, the results thus far indicate that the percentage increase in gray matter was greater than in white.

236. CORTICOID OUTPUT OF HUMAN ADRENAL. James D. Hardy and M. Don Turner.* Dept. of

Surgery, Univ. of Mississippi Med. Ctr., Jackson. At laparotomy timed collections of blood flow were taken from the left central adrenal vein (which represents virtually the entire venous drainage of this organ), and free and conjugated hydrocortisone values were compared with those in systemic blood in 10 patients. The average measured adrenal vein blood flow was 25 cc/min, the average adrenal venous plasma level of free hydrocortisone 224 gamma %, and the average of conjugated hydrocortisone 125 gamma %. In systemic blood the average preoperative values for free and conjugated forms were 5.6 and 7.8 gamma %; 24.0 and 16.7 during operation; and 9.9 and 25.2 gamma % for free and conjugates, respectively, 4 hr after operation. The relative preponderance of conjugated forms postoperatively perhaps reflected the rapid conjugation of the large amount of free hydrocortisone secreted by the adrenal cortices during operation. It is estimated on the basis of plasma values that during operation combined hydrocortisone output of both adrenals was 77 gamma/min, red cell steroid content not considered. The hydrocortisone output by both adrenals was estimated on the basis of plasma values to be 34 mg/24 hr. The methods of Nelson and Samuels and of Bongiovanni were employed. (Army Contract No. DA-49-007-MD-627.)

237. INFLUENCE OF COLD EXPOSURE ON DEVELOPMENT OF MILD THERMAL BURNS IN THE RAT. James Daniel Hardy and L. Zitowitz.* Dept. of Physiology, School of Medicine, Univ. of Pennsylvania, Philadelphia, and the Naval Med. Acceleration Lab., Johnsville, Pa.

On the assumption that cutaneous noxious stimulation indicates the progress of tissue injury in the skin, it was anticipated that following mild thermal burns, which in man are associated with spontaneous pain, a lowering of the skin temperature by 4-5°C would affect the progress of the burn development. To test this hypothesis, second degree thermal burns were produced in rats, Wistar strain, by direct contact in constant temperature water bath at 55°C for 25 sec. Within half an hour after burning, the experimental animals were moved to an environmental temperature of 1-4°C. Control burned animals were maintained at room temperature (25-27°C). After cold exposure the experimental animals were returned to room temperature and their burns were followed in comparison with control animals for a period of 2 wk or until the burns were healed. Experiments were carried out with a cold exposure of 2-, 4-, and 15-day cold exposures. Measurements were made of the areas of inflammation, edema and scab formation. As compared with the control rats, the burns in the cooled rats showed marked inhibition of burn development. The 4-day period of cold exposure seemed to have the maximum inhibitory effect.

238. DETERMINATION OF PULMONARY DIFFUSING CAPACITY FROM CARBON MONOXIDE EQUILIBRATION CURVES. Paul V. Harper, Benjamin Burrows and Frances Dietiker (intr. by J. Garrott Allen). Depts. of Medicine and Surgery, Univ. of Chicago, Chicago, Ill.

In a single compartment model, it is possible to express the diffusing capacity in terms of the wash-in or wash-out curves of carbon monoxide and an inert gas. The use of an exponential approximation is justified by comparison with single breath-cycle calculations similar to those of Gilson and Hugh-Jones over a wide range of tidal volumes when $V_L = FRC + 1/2\ V_T$. This leads to the simple expression: $dP_{CO_A}/dt = K_{N_2}(P_{CO_I} - P_{CO_A}) - K_D P_{CO_A}$, where K_{N_2} is the exponential decay constant for the N_2 wash-out and $K_D = P_B D_{CO}/V_L$. Integrated, this equation gives: $P_{CO_A}/P_{CO_{AO}} = e^{-K_{CO}t}$ for the wash-out curve, where $K_{CO} = K_{N_2} + K_D$. Subjects breathed .1% - .3% CO in air until the $P_{CO_{end\ tidal}}$ became constant. A wash-out of CO and N_2 was then performed; the gases were analyzed in end-tidal samples of each breath by an appropriately calibrated infra-red spectrophotometer and Lilly nitrogen meter. From the CO and N_2 wash-out curves K_{N_2} and K_{CO} were measured using the principal N_2 component. The diffusing capacity was then calculated: $D_{CO} = V_L/P_B\ (K_{CO} - K_{N_2})$. The values obtained appeared to be very reproducible. Seven determinations on 1 normal subject gave a standard deviation of 7%. The sensitivity to small errors in measurement is slight. In 3 normal subjects at rest the values for D_{CO} obtained agreed well with those obtained by Forster's single breath method. We are hopeful that this approach will be useful in the study of pulmonary compartmentation.

239. INTRACORONARY INJECTION IN NON-INFARCTED AND INFARCTED HEARTS. A. Sidney Harris, Richard A. Liptak,* Duncan D. Burford* and Louis A. Toth. Dept. of Physiology, Louisiana State Univ. School of Medicine, New Orleans.

Slow intracoronary injection of potassium chloride, 1-2 mg/kg in the dog evokes ventricular ectopic arrhythmias. This occurs in hearts without infarction. Ventricular fibrillation is a constant danger. The KCl is mixed in a syringe with freshly drawn blood to a total volume of 10 cc. Physiologic saline, isotonic glucose or isotonic sodium lactate similarly administered have not produced ectopic arrhythmias. In infarcted hearts, 1 or more days after occlusion, injections into the peripheral end of the occluded vessel produces results which have striking differences from those of the uninfarcted heart. Physiologic saline, isotonic sodium lactate or isotonic glucose now often produce impressive increases in ectopic activity if preexistent, or cause ectopic activity to appear if not present. The effect is greatest in the first 1 or 2 injections, and diminishes or ceases with repetitions. Sodium lactate has also caused striking remissions of ectopic activity. This is considered a specific effect in contrast with the non-specific effect above. Potassium injections in infarctions appears to differ from isotonic sodium salts and glucose in that ectopic responses remain more repeatable. It is suggested that fluids traversing infarcts acquire excitatory substances and move them to responsive regions. (Supported in part by Grant H-1109 (C4), Natl. Heart Inst., PHS.)

240. EFFECTS OF VARIATIONS IN POTASSIUM CONCENTRATION ON ION TRANSPORT AND BIOELECTRICAL POTENTIALS OF FROG GASTRIC MUCOSA. John B. Harris,* Heddy Frank* and I. S. Edelman. Dept. of Medicine, Univ. of California School of Medicine, San Francisco.

The distal renal tubular cells and the gastric mucosa both secrete hydrogen ions and maintain a negative intraluminal potential as compared with surrounding interstitial fluid. The purpose of this study was to determine whether or not the gastric mucosa would respond to changes in potassium concentration as does the kidney. Net transport of sodium, potassium, chloride and hydrogen ions, volume production and potential difference (p.d.) of the gastric mucosa of Rana pipiens was measured in vitro. The serosa was bathed in buffered, modified Krebs-Henseleit solutions containing 1.0, 4.1 or 8.5 mM/l., KCl.

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Twenty-eight experiments have been completed. Net sodium transport from nutrient to secretory tended to be greatest with low potassium nutrients. Net potassium flux increased with high potassium nutrients. Acidification of the secretory phase was not inhibited by high potassium concentrations since hydrogen ion production averaged 0.4 mEq/hr more with high potassium as compared with low potassium nutrients. Volume production tended to be least with a high potassium nutrient. The p.d. was inversely proportional to the nutrient potassium concentration. High potassium concentrations depressed p.d. to 70% of the control value, while low potassium concentrations produced a 30% rise in p.d. over control values. These data indicate that the response of gastric mucosal cells to potassium loads differs from the renal response.

241. FUNCTION OF BLOOD-PERFUSED DOG KIDNEY AT LOW TEMPERATURES. Rodney B. Harvey. Dept. of Physiology, College of Medicine, Univ. of Utah, Salt Lake City.

The influence of cooling on renal function was investigated by means of an isolated dog kidney perfused with arterial blood by a heparinized "perfusor" dog. This procedure permits the comparison of renal functions of the perfusor dog kept at constant temperature and the isolated kidney, the blood temperature of which was varied from 38.5°-2.5°C. The isolated kidney showed greater reabsorption of H_2O and Na and a lower perfusion pressure than the "perfusor's" kidneys. Cooling depressed the tubular reabsorption of specific substances in different manners. For instance, the sodium concentration difference between urine and plasma was nearly absent below 20°C, while concentration differences of creatinine, inulin, Evans blue, and total solute were always present at the lowest temperature reached. One of many interpretations is that although cooling reduces O_2 consumption and CO_2 production to immeasurably small values, urine formation continues by ultrafiltration with partial reabsorption of water and solutes other than inulin, creatinine and mannitol. There were also marked changes in renal vascular resistance, filtration fraction and renal weight during cooling. All of the measured changes produced by cooling were reversed by rewarming.

242. EFFECT OF ELECTROLYTES ON EXCITABILITY AND ELECTRICAL PROPERTIES OF SINGLE NERVE FIBER OF FROG. Saburo Hashimura* and Ernest B. Wright. Physiology Dept., School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.

Single nerve fibers were dissected from the sciatic nerve of Rana pipiens and mounted for study using the air gap technique of Tasaki. Two nodes were exposed and immersed, one, test node, in a pool of solution which was vaired, and the other, contrast node, in a pool of Ringer's solution. Results: 1) potassium lack: increase in membrane potential of test node, increase in threshold (rheobase) of test node, increase in threshold of contrast node, retarded conduction; 2) potassium excess: decrease in membrane potential of test node, increase in threshold of test node, decrease in threshold of contrast node, decrease to extinction of action potential of test node; 3) sodium depletion: increase of membrane potential of test node, increase of threshold of test node, increase of threshold of contrast node, decrease to extinction of action potential of the test node; 4) calcium lack: no membrane potential change, marked decrease of threshold of test node, no change in threshold of contrast node, no delayed increase in threshold of test node even after 70 min, repetitive firing; 5) excess calcium: no membrane potential change, sharp rise in threshold of test node, no change in threshold of contrast node, retarded conduction; 6) cooling: test node 10° to -1° compared to contrast node 10° constant caused reduced voltage and increased duration of test node action potential. Conduction ceased at 1°C, test node action potential becoming a large local response. It is concluded that excitability rheobase voltage and membrane potential are not directly related.

243. ESTIMATION OF CENTRAL BLOOD VOLUME USING CONSTANT INFUSION TECHNIQUES. Edward W. Hawthorne, Mary Gaspar,* Russell Minton, Jr.,* Sheridan L. C. Perry,* William G. Pogue* and Herman Branson.* Depts. of Physiology and Physics, Howard Univ., Washington, D. C.

In a reasonable hemodynamical model for the dog, it can be shown that the central blood volume is related to the constant rate of infusion through the equation CBV = It/Ceq where CBV is the central blood volume, I is the infusion rate in mg/min, Ceq is the arterial concentration at equilibrium and t is the time required for arterial and mixed venous concentrations to become equal following cessation of the infusion. In a series of experiments with normal dogs, using sodium para-aminohippurate (PAH) as the infusate, the calculated central volumes averaged 310 cc. These values compared favorably with central blood volumes (377 cc) that we obtained by use of the isotope dilution technique of MacIntyre and associates (Circulation 4:552, 1951).

244. HEMODYNAMICS OF MITRAL STENOSIS AND REGURGITATION. Florence Haynes, Paul Novack,* Robert Schlant,* Arthur Phinney* and Lewis Dexter. Depts. of Medicine, Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.

Left heart catheterization data from 37 patients with incapacitating mitral disease have been analyzed. Indicator-dilution methods of Hamilton and of Korner and Shillingford were employed to calculate aortic (AO) and mitral regurgitant (RF) flows. Patients were divided into 3 groups according to mitral stenotic valve area calculated by the Gorlin formula: 1) 13 patients with predominant stenosis (mean calculated valve area 0.8 sq cm, 0.4-1.0); 2) 10 patients with combined stenosis and regurgitation (1.2 sq cm, 1.1-1.5); 3) 14 patients with predominant regurgitation (2.7 sq cm, 1.7-4.7). Mean AO of the groups were 2.2 (1.4-3.3), 2.4 (1.6-3.1), 2.8 (1.8-3.9) l./min/m^2. Mean RF were: 0.3 (0.0-1.2), 1.5 (0.6-1.8), 4.6 (1.1-7.9) l./min/m^2. Mean total left ventricular outputs were: 2.5 (1.4-3.6), 3.9 (2.9-4.9), 7.4 (3.4-11.1) l./min/m^2. Average left atrial mean pressures: 25 (7-40), 24 (15-30), 24 (10-35) mm Hg. Mean mitral diastolic gradients: 22 (10-37), 19 (9-28), 17 (8-27) mm Hg. Mean left ventricular end-diastolic pressures (LV_{ed}): 5 (-7 - 11), 8 (2-14), 9 (0-15) mm Hg. Calculated mitral valve area agreed reasonably well with operative findings. LV_{ed} in all groups was normal or only slightly elevated despite greatly enlarged left ventricles radiologically. Some degree of mitral stenosis was present in all groups. The combination of mitral stenosis and high mitral diastolic flow, occasioned by regurgitation, was responsible for elevation of LA pressure and pulmonary congestion rather than left ventricular failure.

245. HORMONAL EFFECTS ON UPTAKE OF NEUTRAL FAT BY THE PERFUSED LIVER. Murray

Heimberg and H. C. Meng (intr. by E. V. Newman). Dept. of Physiology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Isolated rat livers were perfused with neutral fat emulsions, and disappearance of fat from the medium was measured either turbidometrically, or chemically by means of the hydroxamic acid method for fatty acid esters. It was observed that livers from normal fed female rats took up fat 78% more rapidly than did male livers. Livers from fasting male rats, or from fed alloxan diabetic male rats took up fat at a rate twice that of normal fed male livers. Adrenalectomy markedly elevated the rate of fat uptake by the liver. Cortisone administration returned this rate to normal. The uptake of fat by livers from normal fed animals was not depressed by cortisone treatment, although cortisone reduced the lipid uptake by livers from normal fasting rats. Hypophysectomy, as well as administration of crude anterior pituitary extracts, stimulated the rate of lipid uptake by the liver. Intracellular distribution of fat particles was observed upon histological examination of the perfused liver. The observations suggested that, when the hormone is given to the animal, certain adrenal steroids inhibit neutral fat uptake by the liver in vitro, and that some component of the crude anterior pituitary extract, presumably not adrenocorticotrophic hormone, stimulates this process. The hormonal regulation of neutral fat uptake by the liver may be one important mechanism whereby the animal can alter the fraction of its caloric requirement that it derives from fat.

246. A METHOD OF MEASUREMENT OF THE DYNAMICS OF BONE HEALING. J. H. Heller, H. König,* L. Fox* and A. Torok.* Divs. of Physiology and Exptl. Surgery, New England Inst. for Med. Research, Ridgefield, Conn.

In order to investigate the dynamics of bone healing in vivo, a technique has been devised using the gamma emitting isotope Strontium 85. Sr^{85} has no beta emmission. Since it is expected that strontium or calcium will concentrate heavily at the site of a healing lesion, it was felt desirable to avoid a gamma emitting isotope which also emits a beta particle such as Calcium 47 in order to minimize ionization damage at the fracture. In addition, the 65-day half life of Sr^{85} makes it more useful for study of a lesion throughout the healing process as opposed to the 4.8-day half life of Ca^{47}. Furthermore, the .51 mev gamma of Sr^{85} is much easier to shield against than the 1.3 mev gamma of Ca^{47}. In order to reduce the amount of isotope needed for injection to the absolute minimum, a gamma ray spectrometer is used. The scintillation crystal is shielded by 9 cm of lead and the lesion is measured through an orifice in the shielding which is 2 mm in diameter. A variety of bone lesions are induced in experimental animals. The Sr^{85} uptake at the site of the lesion as well as control values from the normal contralateral limb are taken at daily intervals. Some of the dynamics of bone healing as determined by this method will be discussed. (Supported by a grant from the Gustavus and Louise Pfeiffer Research Fndn.)

247. RESPIRATORY EFFECTS OF PNEUMOTHORAX. Allan Hemingway and Daniel H. Simmons.* Dept. of Physiology, Univ. of Calif. Med. Ctr., Los Angeles.

Pneumothorax has been induced in anesthetized dogs by injecting air into the intrapleural space in steps equal to one half of the functional residual capacity. Respiratory rate, minute volume of respiration, intrapleural pressures, oxygen content and saturation of arterial blood, blood pH and CO_2 content, and percentage of lung collapse for each step of the pneumothorax has been measured. It has been found that the animals have an efficient physiological compensation for pneumothorax, being able to tolerate air injections of 2-3 times the functional residual capacity. Vagotomy and oxygen breathing do not appreciably change the tolerance to pneumothorax. As the amount of injected air in the intrapleural space is progressively increased, hyperventilation occurs reaching a maximum with a pneumothorax of 1.5-2.0 times the FRC. The hyperventilation is greater with air breathing than with O_2 breathing and during air breathing the hyperventilation rises as the O_2 saturation falls, indicating that the sinoaortic chemoreceptors are partly responsible for the hyperventilation. When pneumothorax is induced during oxygen breathing, a well defined respiratory acidosis occurs. The intrapleural pressure changes which occur consist of a progressive increase in inspiratory-expiratory differential. The expiratory pressure soon becomes positive and the positive expiratory pressure continues to increase as the pneumothorax volume increases. The rising expiratory pressure is accompanied by powerful expiratory movements. The negative inspiratory pressure at first becomes less negative and then more negative as the lung collapse is increased.

248. RATE OF CHANGE OF SKIN TEMPERATURE AND TEMPERATURE SENSATION. E. Hendler and James Daniel Hardy (intr. by Charles F. Gell). Air Crew Equipment Lab., Naval Air Materiel Ctr., Philadelphia, and Dept. of Physiology, Med. School, Univ. of Pennsylvania, Philadelphia.

Subjects were exposed over a prolonged time interval to changes in radiant heat applied to a central area of the forehead. Simultaneous recordings of skin temperature of the irradiated area were obtained using a very sensitive radiometric apparatus. Reports of temperature sensation arising from the stimulated area were made at 10-sec intervals. Rapid changes in skin temperature rate which lasted only a short time were unable to evoke consistent sensation reports when the general level of skin temperature was close to its normal value. Under similar conditions, longer lasting rates of change in skin temperature were accompanied by temperature sensations in a manner consistent with Weber's original hypothesis on the nature of the adequate stimulus for temperature sensation. Applications of radiation producing constantly increasing skin temperature rates of change resulted in consistent reports of warmth, although the duration of these applications far exceeded the adaptation time as usually determined for the same change in skin temperature. Preliminary heating or cooling of the forehead skin resulted in a shift of threshold for the rate of change of skin temperature necessary to evoke sensation. The relationship of these findings to the "adequate stimulus" is discussed.

249. FUNDAMENTAL PROPERTIES OF RAT HEART TISSUE IN EXPERIMENTAL RENAL HYPERTENSION. J. P. Henry,* W. V. Whitehorn and T. M. King.* Dept. of Physiology, College of Medicine, Univ. of Illinois, Chicago.

As part of a more general investigation of physiological properties of cardiac muscle in various types of hypertrophy, male albino rats were rendered hypertensive by application of latex bags to their kidneys. Blood pressure, body weight, heart weight and adrenal weight were determined on 16 experimental

animals and compared with values on 24-sham operated controls. Refractory period, excitability to square wave stimuli and active and passive length-tension curves were determined in vitro on left ventricular columnae carnae in bicarbonate buffered Ringer's solution. Hypertensive rats showed significant elevation of heart rate, heart weight, heart-body weight ratio, and adrenal weight. Water content of hypertensive cardiac tissue was slightly increased. No change was observed in excitability or refractory period. Mean active tension at given resting length tended to be greater in experimental animals, but this difference was not statistically significant. Passive length-tension curves indicated increased resistance to stretch in muscle from hypertensives. These results suggest that cardiac hypertrophy associated with experimental renal hypertension is not accompanied by significant changes in excitability and contractility of isolated cardiac tissue.

250. EFFECT OF COLD ACCLIMATION ON THICKNESS AND MITOTIC INDEX OF EPIDERMIS. O. Heroux (intr. by J. S. Hart). Natl. Research Council, Ottawa, Canada.

Histological sections revealed that, after a 4-wk continuous exposure to +6°C, the Malpighian layer of the epidermis in white rats was twice as thick as before acclimation in the ear and in the tail but not in the plantar region or in skin regions well covered by fur such as those on the back or the belly. Such thickening of the epidermis, at least in the ear, was not found in white rats cold acclimated either individually by intermittent exposure to 30° and 6°C or collectively by maintaining 10 of them together in a large cage kept outside during the winter, nor in wild Norway rats capture during the winter. In rats continually kept at +6°C for 4 wk, the surface temperature of the skin measured at 6°C over the plantar region, the tail and the back was observed to be respectively: 18°, 16° and 33°C in comparison to 33°, 33° and 36°C in 30°C rats measured at +30°C. A study of the mitotic index done on the same rats also revealed twice as many cells in division in the ear and the plantar region after as before acclimation. Calculation of the mitotic duration and renewal time in these epidermis samples should determine whether or not the higher mitotic index observed in these tissues having a 15-18°C surface temperature actually reflected an increased tissue turnover rate.

251. BIOSYNTHESIS OF RIBOSE IN HeLa CELL GROWN IN TISSUE CULTURE. Howard H. Hiatt (intr. by H. L. Blumgart). Dept. of Medicine, Beth Israel Hosp. and Harvard Med. School, Boston, Mass.

Synthesis of nucleic acid ribose via the pentose phosphate pathway in microorganisms, the chick and the rat has been suggested by several investigators. Decarboxylation of carbon 1 of hexose appears to be the predominant route of synthesis in bacteria, but in the chick and the rat substantial ribose production occurs via the transketolase reaction, i.e. condensation of a fragment derived from the first 2 carbon atoms of hexose phosphate with a triose phosphate acceptor. To study ribose synthesis by mammalian cells in tissue culture, HeLa cell was grown in the medium described by Eagle (J. Biol. Chem. 214:839, 1955), supplemented with $NaHC^{14}O_3$, glucose-1-C^{14}, glucose-2-C^{14}, glucose-U-C^{14} or ribose-1-C^{14}. Degradation of RNA ribose was carried out by microbiological (L. pentosus) and chemical methods. All C^{14} substrates were incorporated into cellular fractions. In a typical study the ribose from cells grown in the presence of glucose-1-C^{14} had 54 and 34% of its radioactivity in carbons 1 and 5, respectively. The data indicate substantial ribose biosynthesis in HeLa cell via the transketolase reaction. Thus, the route of ribose synthesis in this human tumor cell grown in tissue culture corresponds to that observed in in vivo mammalian studies. The large isotope concentration in C-5 of ribose is considered to reflect cleavage of hexose-1-C^{14} to triose phosphates and subsequent isomerization of the 3-carbon fragments. (Supported by the Jane Coffin Childs Fund and PHS Fund C-2673.)

252. HEPARIN, A BIOLOGIC ION EXCHANGER IN PROTECTION VS. NOXIOUS AGENTS. R. D. Higginbotham* and Thomas F. Dougherty. Dept. of Anatomy, Univ. of Utah College of Medicine, Salt Lake City.

Heparin can bind histamine or 48/80 in vitro and 48/80 can inhibit this histamine binding (Werle and Amann, Klin. Wschr. 34:624, 1956). In vivo, heparin is found in association with histamine and can bind 48/80. Histamine releaser activity of 48/80 may involve an ion exchange reaction. The possible role of heparin as an ion exchanger was investigated in vitro. A complex of the basic dye, toluidine blue, with heparin was rapidly dissociated in the presence of graded amounts of 48/80 as the heparin formed a new complex with 48/80. Amount of dye released was related to 48/80 concentration employed. Histamine releasers; stilbamidine and polymyxin as well as antibiotics; neomycin, viomycin and streptomycin also replaced toluidine blue in this system. Enhanced tolerance of heparinized mice for 48/80 has been reported from this laboratory (Proc. Soc. Exper. Biol. & Med., 92:493, 1956) and was related to the in vivo binding of 48/80 by heparin with the subsequent fibroblastic ingestion of the 48/80-heparin complex. Heparin, given prophylactically or therapeutically, can also enhance the tolerance of mice for stilbamidine, polymyxin, neomycin, viomycin or streptomycin. Fibroblastic ingestion of heparin in complex with these noxious substances has been observed. The protective nature of "micellophagosis," as previously postulated (Fed. Proc. 14:232, 1955), is supported by these observations that acidic mucopolysaccharides may have an ion exchange function in the tissues which, under certain conditions, acts as a local means of detoxification. (Supported by Grant #DA-49-007-MD-130, Dept. of the Army, and Burroughs Wellcome and Co., Inc.)

253. MEASUREMENT OF ELECTRICAL POTENTIAL ACROSS ISOLATED HUMAN GASTRIC MUCOSA. N. C. Hightower, Jr. Scott and White Clinic, Temple, Tex.

Twenty-five specimens of fresh human gastric mucosa were obtained at the time of gastrectomy and immediately placed in an oxygenated modified Krebs solution at 4°C for transportation to the laboratory. The mucosa was rapidly and gently dissected from the submucosal tissues and mounted as a septum in an incubation chamber and placed in a constant temperature bath at 37.5°C. The incubation chambers were made to expose 1 or 2 cm^2 of mucosa to oxygenated electrolyte solutions in the chamber. The electrical potential was measured by calomel half-cells connecting agar-KCl electrodes to a pH meter. After approximately 20 min, during which time the electrolyte solutions were changed frequently to wash out anesthetic agents, the mucosa attained its maximal electrical potential state. Maximal potentials varied from 10-40 mv with an average of 24 mv. The

maximal potential was maintained at a plateau for 40-60 min and then began to decline slowly. After an additional 120 min, the potential was reduced to 3-5 mv. During the plateau period of maximal potential, the effects of anoxia, temperature, the presence or absence of substrate (glucose) and alterations in the composition of the electrolyte media were studied. In addition, the electrical potential was measured across the mucosa of the sigmoid and transverse colon.

254. INSULIN COMA AND HEMATO-ENCEPHALIC EXCHANGE. Williamina A. Himwich. Galesburg State Research Hosp., Galesburg, Ill.

Rabbits maintained in deep insulin hypoglycemia (blood sugar less than 20 mg %) for 4 hr or more with light barbiturate anesthesia and artificial respiration show a decrease of about 40% of the free glutamic acid and a slightly greater fall in free glutamine of the brain. The glutamic acid and glutamine were determined chemically; the experimental set-up was that described by Sullivan and Strong (Am. J. Physiol. 181:43, 1955) and Himwich and Sullivan (J. Nerv. & Ment. Dis., in press). Electroencephalograms in such hypoglycemic animals do not show normal electrical activity in the limbic lobe or motor cortex. The subcutaneous administration of L-glutamic acid, 2 gm/kg as sodium glutamate at pH 7.0, restores the free glutamic acid of brain toward normal values, but does not restore the EEG pattern. Glutamine is scarcely affected. In such animals there is little or no hyperglycemic effect of glutamic acid. It is suggested that although the brain in the absence of glucose uses free glutamic acid, an excess of the amino acid cannot restore the brain to normal in hypoglycemia. In insulin coma the hemato-encephalic exchange of glutamic acid is increased as indicated by the increase in free glutamic acid when sodium glutamate is given.

255. GRAM NEGATIVE ENDOTOXIN AND RENAL FUNCTION. L. B. Hinshaw,* C. H. Carlson,* G. M. Bradley* and M. B. Visscher. Depts. of Physiology, Physiological Chemistry and Medicine, Univ. of Minnesota, Minneapolis.

Studies were made on dog kidneys perfused by a constant pressure system from a heart-lung preparation. Creatinine and paraamino hippurate (PAH) extraction ratios were measured, as were urine flow, renal blood flow and kidney weight. Clearance rates were calculated. The kidneys were supplied with a constant infusion of Pitressin to maintain an elevated urine specific gravity. In this preparation PAH extraction ratios remained high during control periods. After administration of E. coli endotoxin in doses lethal to dogs the blood flow rate at constant perfusion pressure fell temporarily, as did the kidney weight. The PAH extraction ratio regularly fell progressively while the creatinine extraction ratio fluctuated in an inconsistent way. The urine specific gravity fell. After the renal blood flow returned to pre-injection levels the indices of renal tubular function, PAH clearance and urine specific gravity, did not improve but rather deteriorated. Urine volume changes were not regular. It was concluded that in the dog kidney perfused at constant pressure from a heart-lung preparation E. coli endotoxin produces a severe impairment of renal tubular function. These observations indicate that arterial hypotension is not a necessary factor in the renal impairment in endotoxin shock.

256. COMPARATIVE PHYSIOLOGY OF ESTROGENS. Frederick L. Hisaw, Jr. (intr. by F. L. Hisaw). Harvard Univ., Biological Labs., Cambridge, Mass.

Five natural occurring estrogens, estradiol-17B, estrone, estriol, equilin and equilenin and one synthetic estrogen, diethylstilbestrol, were tested for uterine growth and fluid imbibition by using a modification of the Astwood (1938) method. Twenty-two-day old rats weighing 40-50 gm were used and comparisons were based on responses following a single subcutaneous injection. The order of overall effectiveness for growth (30th-48th hr) is diethylstilbestrol, estradiol, equilin, estrone, equilenin and estriol. For fluid imbibition, the order of potency varies depending upon dosage and time. As dosage is increased the time required for maximal fluid imbibition decreases, as determined by total weight, so that a value occurring at 6 hr or later will occur at 4 hr or earlier. Natural estrogens superior for growth (estradiol, equilin) are less effective than others (estriol, equilenin) for fluid imbibition, and vice versa; the two responses being separate and distinct so that one can occur independent of the other. However, diethylstilbestrol and equilin at high doses are capable of producing both growth and fluid imbibition simultaneously. Physiologically, natural estrogens seem to be associated at least in pairs in which one member is considerably stronger than the other in promoting growth. These differences of degree give each estrogen an individual physiological specificity.

257. URETERAL REFLEX MODIFYING RENAL FUNCTION. Elliott Lee Hix (intr. by Irvin M. Korr). Dept. of Physiology and Pharmacology, Kirksville College of Osteopathy and Surgery, Kirksville, Mo.

The surgical exteriorization of one or both ureters into a flank skin-flap in the dog has proven satisfactory for the study of unilateral renal function in the conscious animal (Hix, J. Appl. Physiol. 8:114, 1955). With this preparation slight irritation of the ureter reflexly modifies ipsilateral renal function by decreasing ERPF and GFR. Nupercaine applied to the lower third of the ureter blocks this reflex indicating the presence of sensory nerve fibers which communicate reflexly with renal vasoconstrictor fibers. This is always unilateral and ipsilateral. Emotional stress, superimposed on ureteral irritation, produces a reduction in ipsilateral ERPF and GFR as compared to the contralateral control response. While ureteral irritation alone may not always induce an observable decrease in ERPF and GFR, on subjecting the animal to stress the ipsilateral kidney still shows an exaggerated response. It appears that efferent renal nerve pathways are facilitated by ureteral irritation. This pattern of uretero-renal reflexology can be induced, controlled and pharmacologically blocked in the conscious animal under good experimental conditions. These observations suggest that a subtle and/or chronic focus of ureteral or other irritation may act, through facilitating renal nerve pathways, to channelize autonomic activity into these efferent renal pathways, thus, exposing the kidney to exaggerated impacts from environmental stress factors. (Supported by grants from the Natl. Heart Inst. (H-1632) and the American Osteopathic Assoc.)

258. REFRACTORINESS OF CARDIAC MUSCLE. Brian F. Hoffman and P. F. Cranefield. Dept. of Physiology, State Univ. of New York, Downstate Med. Ctr., New York City.

The apparent duration of the absolute refractory period of cardiac muscle depends in part on the duration and maximum strength, as well as the polarity, of the test pulses employed (Brooks, Hoffman, Suckling and Orias. Excitability of the Heart, New York:

Grune & Stratton, 1955). The physiological significance of measurement of the refractory period by usual techniques is thus open to question. A comparison of refractoriness to test stimuli and to propagated action potentials has been made. The absolute and effective refractory periods of a single Purkinje fiber from the dog's right ventricle have been determined, first, by means of rectangular current pulses of 10-20 msec duration applied through an intracellular microelectrode and, secondly, by means of propagated action potentials originating in an attached papillary muscle and reaching the Purkinje fiber at various times during its phase of repolarization. The results indicate that the earliest propagated responses elicited by either method of stimulation arise from similar levels of membrane potential and thus give effective refractory periods of comparable duration. Non-propagated responses to both types of stimuli are also similar. Anodal current pulses, however, were found to stimulate at any time during repolarization if of adequate duration and strength. The expected similarity of refractory periods determined by means of propagated action potentials and by cathodal stimuli of moderately long duration suggests that the latter give an adequate indication of recovery of the ability to develop a propagated response.

259. ESTROGEN RESPONSE OF MAMMARY GLAND IN ADRENALECTOMIZED GUINEA PIGS. E. Otto Höhn (intr. by A. W. Downs). Dept. of Physiology, Univ. of Alberta, Edmonton, Canada.

It has been suggested that the reason that guinea pigs do not, like most laboratory mammals, require progesterone as well as an estrogen for full mammary gland development is due to progesterone production in the guinea pig adrenal. To test this hypothesis, estrone was given by inunction of an alcoholic solution over the glands of adrenalectomized males, given extra sodium chloride. Survival after adrenalectomy is adversely affected by estrone, shown by comparison with a group of non-estrone treated adrenalectomized animals. Nevertheless 8 adrenalectomized animals survived estrone treatment for 13-28 days. Controls with intact adrenals show a fair degree of mammary alveolar development after 14 days estrone treatment. The adrenalectomized animals showed only duct development with rare isolated alveoli instead of the clusters seen in controls. The findings are thus in agreement with the hypothesis. The adreno-cortical contribution to the estrone stimulation is however not necessarily progesterone, as other cortical steroids can, in other species, act as progestogens. The adverse effect of estrone on survival after adrenalectomy is surprising since estrogens are reported to have mineralo-corticoid like activity on electrolyte balance.

260. VARIATIONS IN VENTILATION BLOOD FLOW RATIOS DURING ARTIFICIAL RESPIRATION. D. A. Holaday, J. Israel, E. K. Williams and M. J. Frumin (intr. by Robert C. Darling). Depts. of Anesthesiology and Medicine, Columbia Univ. College of Physicians and Surgeons, and Presbyterian Hosp., New York City.

Arterial O_2 saturation and CO_2 tension of anesthetized patients were determined with a manometric method and glass electrode before and during artificial respiration produced by 3 different intermittent positive pressure devices. A mouth-to-mask resuscitation device (Elam et al. New Eng. J. Med. 250:749) produced adequate CO_2 excretion in 4 of 6 subjects ventilated with room air, but provided adequate oxygen saturations in only two. The saturation of one subject fell to 76% when the P_{CO_2} was 39 mm Hg; in another the saturation was 87% at a P_{CO_2} of 30 mm Hg. A mechanical cough machine, or exsufflator, was used to ventilate 7 patients emerging from anesthesia (Am. J. Surg. 90:637). During exsufflation with room air, the average P_{CO_2} was reduced from 48-38 mm Hg, whereas the average saturation rose only 2.2% from 87-89%. A mechanical respiration in which the inflating pressure is servo-controlled by the end tidal P_{CO_2} (Frumin & Lee, J. Lab. Clin. Med. i.p.) was used to maintain P_{CO_2} between 26 and 44 mm Hg with gas mixtures containing 21-50% oxygen. Six patients were encountered whose saturations fell to or below 91%. In none of the patients was there known to be pre-existing lung pathology nor was there direct surgical interference with lung function. Possible contributing factors resulting from anesthesia and surgical operation as well as from artificial respiration will be discussed.

261. DEFECTIVE PLATELETS AS CAUSE OF DEFICIENT GENERATION OF THROMBOPLASTIN IN A FAMILY OF HEMOPHILICS. R. R. Holburn,* R. T. Carroll* and L. M. Tocantins. Cardeza Fndn., Jefferson Med. College, Philadelphia, Pa.

Defective platelet activity was the principal abnormality accounting for the delayed and insufficient thromboplastin generation in a group of patients with characteristically inherited hemophilia. Venous blood clotting times were prolonged and there was slightly decreased prothrombin and Ac-globulin. Antithrombin and antithromboplastin activity were normal. Normal platelets restored the clotting time of the patients' plasma to normal; equal concentrations of the patients' own platelets did not. Increasing amounts of washed normal platelets restored to normal the thromboplastin generation system containing patients' plasma and serum. Increasing the concentration of the patients' platelets did not alter their effectiveness in the normal or abnormal systems. Slow and insufficient thromboplastin was generated when patients' serum was used in the system, or when a serum was employed produced by clotting normal platelet-poor plasma with the patients' platelets. Thromboplastin generation was normal when serum from the patients' plasma clotted with normal platelets was used. Platelet cofactor activity of the patients' plasma was increased, unlike Hemophilia A and B. Patients' plasma was superior to normal plasma in promoting coagulation of grade III Hemophilia A plasma. Addition of patients' plasma to Hemophilia B plasma (1-1) resulted in normal clotting rate. Patients' plasma had no clot delaying action on normal plasma. One hundred ml of platelet-poor normal plasma intravenously (single or multiple daily infusion) did not alter the patients' blood or plasma clotting time and thromboplastin generation. A single infusion of plasma rich with platelets brought the patients' plasma coagulation and thromboplastin generation to normal for at least 3 days.

262. LIPEMIA CLEARING FACTOR INHIBITOR IN NORMAL PLASMA. Charlotte Hollett* and H. C. Meng. Dept. of Physiology, Vanderbilt Univ. Med. School, Nashville, Tenn.

We have reported that normal fasting plasma inhibited the clearing activity of postheparin plasma, purified clearing factor and pancreatic lipase. The inhibitory substance has now been purified with an increase in specific activity by 1200-fold. The physico-chemical properties of the inhibitor are: 1) non-dialysable; 2) heat stable; 3) inactivated by acid hydrolysis; 4) not digested by trypsin or

chymotrupsin; 5) total reducing substances, 3.0 mg/100 mg; 6) Hexosamine, 3.0 mg/100 mg; 7) total proteins, 41.5% and no non-protein nitrogen detected. These data suggest that it is a glycoprotein. The purified inhibitor depressed the clearing activity and release of unesterified fatty acids by postheparin plasma and pancreatic lipase by 34 and 40% in 2 hr, whereas the clearing by purified clearing factor was almost completely inhibited. It seems that the purified clearing factor is more sensitive to the purified inhibitor than postheparin plasma or pancreatic lipase. However, it is not known to what extent the inhibitor is specific for the purified clearing factor. Studies relating to the physiological significance of the inhibitor are in progress.

263. FLOW OF FLUIDS THROUGH COLLAPSIBLE TUBES. J. P. Holt, J. Allensworth,* D. Collins* and J. Diana.* Inst. for Med. Research, Univ. of Louisville, Louisville, Ky.

In order to understand the factors regulating the flow of blood through veins, experiments have been carried out in a model, studying the flow of fluids through a long, thin-walled "collapsible" rubber tube. When such a collapsible tube is held so that its longitudinal axis is vertical, with the upper end connected to a rigid tube through which the fluid enters, the collapsible tube collapses throughout its entire length. The rate of flow (ml/sec) from the rigid tube is unaffected by the presence of the collapsible tube. The cross-section of the collapsible tube is constant throughout most of its length for any given flow, and the mean velocity of flow (cm/sec) is the same through all cross sections. As the flow per unit of time through the collapsible tube is increased the mean flow time (sec) is decreased, the cross section is increased and the mean velocity of flow (cm/sec) is increased. With the flow (ml/sec) through the tube remaining constant, an increase in the viscosity of the fluid, or a decrease in the length of the tube, causes an increase in cross section and a decrease in the mean velocity of flow (cm/sec). The lateral pressure at all points throughout the length of the collapsible tube is atmospheric and independent of the rate of flow (ml/sec) through the tube. As a collapsible tube is tilted from the vertical toward the horizontal, keeping the flow (ml/sec) constant, the cross section of the tube increases. An equation will be discussed. (Supported in part by grants from the American, Kentucky, and Louisville Heart Assocs.)

264. SIMPLIFIED TUBELESS METHOD FOR GASTRIC ACIDITY. J. M. Holthaus, F. L. Humoller, and J. R. Walsh (intr. by A. L. Dunn). Med. Research Lab., VA Hosp. and Creighton Univ. School of Medicine, Omaha, Neb.

A tubeless method for the determination of gastric acidity has been proposed by Segal (Gastroenterology 16:380, 1950) as a substitute for the classical intubation method. Comparative studies by other investigators have demonstrated the merit of the tubeless method. The method should gain further acceptance if it could be modified so that it could be used as a bedside test without requiring the use of a dark room and/or a source of ultraviolet light. In the method as finally adopted the subject is prepared as in the Segal method. Quinine is detected in the urine specimens by adjusting the pH of an aliquot of each urine sample to about 8, saturating the urine with ammonium sulfate. Next a small amount of dye, such as tetrabromophenolphthalein ethyl ester, and 1 ml of benzene is added. After vigorous shaking, to extract the quinine-dye complex, the color of the benzene layer is noted. Varying intensities of the red color of the dye-quinine complex in the benzene layer indicate the presence of gastric acidity. The complete absence of any red coloration signifies a negative test. In all cases of proven achlorhydria negative results were obtained, while in all cases where gastric acidity was established by the intubation method a positive result was obtained with varying degrees of color intensity.

265. TOTAL AND PARTIAL GAS TENSIONS OF HUMAN URINE. Suk Ki Hong* and Hermann Rahn. Dept. of Physiology, Univ. of Buffalo School of Medicine, Buffalo, N. Y.

Normal urine samples were collected at random under oil. Determinations were made of the total gas tension as well as the partial tension of O_2, CO_2 and N_2 using a modification of Krogh's method (Aksnes and Rahn, 1956). The total gas tension averaged 72 mm Hg below atmospheric pressure. This difference is believed to result from a drop in O_2 tension along the kidney tubule which is greater than the corresponding rise in CO_2, while the N_2 tension remains constant. The urine O_2, CO_2 and N_2 tensions averaged 14, 63 and 550 mm Hg, respectively, and may represent the gas tensions of the tubular tissue. These latter values may be compared with those reported for the renal venous blood: namely, 60, 41 and 560. During the peak of water diuresis induced by the ingestion of a liter of water the O_2 tension increased to 26 while the CO_2 tension fell to 48 mm Hg. Ingestion of 400 cc of 4% NaCl solution resulted in a fall of O_2 tension to 6 and a rise in CO_2 to 80. The N_2 tension remained virtually unchanged during these diuresis experiments. Ingestion of 20 gm of $NaHCO_2$ in 3 subjects yielded CO_2 tensions averaging 110 mm Hg without significant changes in O_2.

266. CONJUGATION OF m-AMINOPHENOL BY TISSUE SLICES FROM COLD-EXPOSED RATS. A. Horita and M. E. Denison (intr. by A. D. Keller). Physiology Dept., Army Med. Research Lab., Fort Knox, Ky.

A greater susceptibility of male rats to cold exposure over that of female rats as determined by survival has been previously demonstrated (Denison and Zarrow, Am. J. Physiol. 186:471, 1956). It seems probable that this sex differential is due to a detrimental effect of androgen and is specifically due to formation of a compound which is toxic to the animal. Conjugation is a known mechanism of detoxication of compounds containing free -OH groups, therefore the conjugating capacity of tissue slices from cold-exposed rats was investigated. m-Aminophenol was used as the substrate, and the amount of conjugated m-Aminophenol was determined spectrophotometrically after 1 hr of incubation of liver and kidney slices in a modified Krebs-Ringer-Bicarbonate buffer. The conjugating capacity of tissue from intact and castrated male and female rats kept at room temperature and exposed to cold was determined. Liver tissue from male rats exposed to cold for 15 days had increased its conjugating capacity approximately 100%, whereas the amount of m-Aminophenol conjugated by tissue from female rats after the same period of cold exposure was no different than that formed by tissue from control animals.

267. ROLE OF ALTERED PULMONARY HEMODYNAMICS IN THE REFLEX TACHYPNEA OF MULTIPLE MINUTE PULMONARY EMBOLISM. Alan D. Herres* and Theodore Bernthal. Dept. of Physiology, Med. College of South Carolina, Charleston.

Rapid shallow breathing identical in pattern with that classically associated (Dunn) with diffuse bilateral lung embolization was regularly elicited when the embolizing injections were confined to one lung or to a single lung lobe. This tachypnea was abolished by blocking the vagi. Emboli were glass beads (diameters 10-75 μ) injected into pulmonary artery branches of closed-chest anesthetized dogs during spirometric recording of breathing. Although tachypneagenic embolizations were often unattended by systemic arterial blood pressure changes, pulmonary arterial blood pressures invariably rose 19-100%. A possible role of the pulmonary arterial hypertension in initiating the reflex tachypnea was investigated: While recording pressure pulses in the right pulmonary artery (indwelling catheter and electromanometer), the left pulmonary artery was temporarily occluded (loop surrounding artery branch and manipulated from outside closed chest). This raised the pressures in the unoccluded right branch by 23-47% but did not elicit tachypnea. Continued recording of right pulmonary arterial pressures while embolizing the right lung sufficiently to elicit tachypnea showed a rise but this was not materially greater than that during the left artery occlusion which had not produced tachypnea. Furthermore, reducing arterial pressure in an embolized lung to zero (occlusion of pulmonary artery branch) did not alter the tachypnea. Thus, tachypnea was not produced by increased pulmonary arterial pressure alone in the absence of embolization nor was it abolished by reversing the increased pulmonary arterial pressure associated with embolism.

268. AN ESTIMATE OF THE ACTIVE TRANSPORT SODIUM POOL. T. Hoshiko and Hans H. Ussing (intr. by Gordon L. Farrell). Dept. of Biological Isotope Research, Zoophysiological Lab., Univ. of Copenhagen, Copenhagen, Denmark.

A kinetic approach to the estimation of the size of the sodium pool associated with active transepithelial sodium transport has been attempted. The sudden addition of Na-24 to the solution bathing one side of frog skin results in a steady increase in the tracer flux to a final steady state value. The time course of this build-up of the Na-24 flux follows a single exponential for both in- and outflux. This is consistent with the idea that the passage of the sodium is delayed by just one compartment. The build-up half-times for toad urinary bladder and frog skin range from 2-5 min, and is not accountable as diffusion delay through the connective tissue. The half-time for frog skin connective tissue washout is 1 min or less. The steady state influx divided by the time constant of the build-up curve estimates the minimal amount of sodium in the pool, if only one pool is responsible for the delay. The calculated sodium pool size for urinary bladder and skin are in the same range, from 0.01-0.42 uEq Na/cm^2 (median, 0.06). This amount far exceeds any imaginable as residing in the 200 Å thick basement membrane which was proposed by Linderholm (Acta Physiol. Scand. 31:36, 1954) and others as the site of active sodium transport. Therefore the pool is most probably in the epithelial cells, and the actively transported sodium passes through and not between the epithelial cells.

269. IODIDE METABOLISM OF THE DOG STOMACH. G. L. Howell* and L. Van Middlesworth. Dept. of Physiology, Univ. of Tenn., Memphis.

Gastric clearance of plasma iodide and chloride was determined in 44 experiments on 4 Heidenhain pouch dogs. These studies were made under control conditions and after administration of 1) drugs known to interfere with the iodide concentrating mechanism of the thyroid ($NaClO_4$ 15 mg/kg, NaSCN 75 mg/kg, and NaI 60-100 mg/kg), 2) drugs known to influence the acid secretions of the stomach (Diamox 50 mg/kg and histamine 3 mg), and 3) combinations of these. The results were qualitatively similar to those previously reported on anesthetized dogs (Fed. Proc., 1956). They confirm the impression that iodide was secreted by at least 2 mechanisms a) a specific iodide trap and b) a mechanism in common with chloride. Although the basal iodide clearance was 30 times the basal chloride clearance; $NaClO_4$ depressed the iodide clearance to equal the chloride value. When histamine was injected after perchlorate, the chloride and depressed iodide clearance increased together. Diamox, which blocked acid secretion, inhibited histamine effects upon both anions but the iodide clearance remained 25 times chloride clearance. This active iodide metabolism of the stomach suggests that there may be a physiologic function in secreting iodide. To investigate this possibility dialized milk protein was placed in the Heidenhain pouch. After 2 hr the pouch contents were shown by electrophoresis to contain freshly iodinated protein. This demonstrates possible iodination capacity of the gastric mucosa. (Supported by grants from Atomic Energy Comm. and the PHS.)

270. INHIBITORY POTENTIALS FROM CRUSTACEAN MUSCLES. G. Hoyle* and C. A. G. Wiersma. California Inst. of Technology, Pasadena.

Fatt and Katz (J. Physiol. 121:374, 1953) have reported that in the opener muscle of the hermit crab Eupagurus and the flexor of the dactylopodite of the shore-crab Carcinus maenas, they could obtain membrane potential changes during inhibitory nerve stimulation. These were observed with an internal electrode when the membrane potential was displaced from the resting level by passing current across the membrane. They were negative (polarizing) when the potential was lowered, positive (depolarizing) when it was raised. In the present investigations a number of crustacean inhibitory nerve-muscle preparations are being studied. Similar results to those found by Fatt and Katz have been obtained in certain muscle fibers. In contrast to their observations, however, muscle fibers of various species have been found which show potential changes during inhibitory nerve stimulation at the penetration (resting) membrane potential. They are negative in sign and during repetitive stimulation lead to an enhanced membrane potential. This may decay rapidly or in some cases extremely slowly. Single inhibitory potentials are less than 1 mv, but during repetition summate to raise the membrane potential by as much as 15 mv. The rates of both the development and the decline of the inhibitory potentials varies from fiber to fiber of the same species and also between species.

271. ROLE OF SYMPATHETIC NERVOUS SYSTEM IN CHEMICAL REGULATION OF HEAT PRODUCTION. A. C. L. Hsieh* and L. D. Carlson. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle.

When muscle activity is blocked by curare, only cold-adapted rats can increase heat production sufficiently to maintain body temperature when a test room is cooled from 30°-5°C. The role of the sympathetic nervous system in this response was tested with injections of adrenaline and noradrenaline and of sympatholytic and autonomic blocking drugs. The calorigenic effect of adrenaline is enhanced in cold-adapted animals, and noradrenaline, which has little calorigenic effect in the warm-adapted animal, has a

marked effect in cold-exposed animals. The ganglionic blocking agent hexamethonium chloride and the adrenolytic agent piperoxane hydrochloride prevented the increase in oxygen consumption otherwise observed in curarized, cold-adapted rats exposed to cold.

272. VITAMIN B_{12} DEFICIENCY AND HYPERCHOLESTEROLEMIA. Jeng M. Hsu and Bacon F. Chow (intr. by A. M. Baetjer). Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.

Experiments were conducted to study the effect of vitamin B_{12} deficiency on cholesterol levels of serum and adrenals in rats and chicks. a) Adult rats: 12 male and 6 female rats were placed on a soybean-sucrose diet, deficient in B_{12} (BSD). A like number of rats of both sexes was offered the same diet supplemented with B_{12} (100 μg/kg) (SBSD). Eight or 10 wk later, serum total cholesterol concentrations were measured and found to be 77.6±1.2 vs 67±1.8 for the deficient and B_{12}-treated males; 89.2±6.1 vs 56.2±4.0 mg % for the deficient and B_{12}-treated females, respectively. b) Young rats: weaning female rats from mothers placed on B_{12}-deficient diet during lactation were divided into 2 groups. One group received the BSD while the other, SBSD. Four wk later, the total cholesterol levels in the adrenals of all animals were determined; 4.8±0.6 and 2.6±0.4 mg % of wet tissue for B_{12}-deficient and treated rats, respectively. c) Young chicks: eight New Hampshire chicks were fed a high fat B_{12}-deficient diet. Another 8 were kept on this diet supplemented with B_{12} (100 μg/kg). After 5 wk, the total cholesterol concentrations in the adrenals and sera of the B_{12}-deficient chicks were found to be higher than those of the controls; being 145±9.0 vs 117±6.7 mg % for serum; 3.6±0.2 vs 2.9±0.2 mg % of wet tissue for adrenals. These results show that vitamin B_{12} deficiency induces hypercholesterolemia. The possible role of vitamin B_{12} in cholesterol metabolism will be discussed.

273. SINGLE UNIT ACTIVITY IN VISUAL CORTEX OF THE UNANESTHETIZED CAT. David H. Hubel (intr. by M. G. F. Fuortes). Dept. of Neurophysiology, Walter Reed Army Inst. of Research, Washington, D. C.

Single unit recordings have been made from cerebral cortex in chronic cats. The unanesthetized animal is restrained only by a chest harness, with the head entirely free, and a hydraulic system is used to position a tungsten microelectrode. Isolated spikes have been observed from the visual cortex for periods of the order of 1 hr during the waking state and natural sleep. Lights and moving objects have been used for stimuli, and the effects of arousal and eye opening and closing have been observed. Most of the cells recorded from show brisk spontaneous activity which is modified by the stimuli. The modification, which may be brief or prolonged throughout the stimulus, may consist of an increase or decrease of activity, and may involve an alteration in the degree to which spikes are grouped. Thus many varieties of responses are seen, including the 4 described by Jung et al. (EEG Clin. Neurophysiol. Suppl. 4:57, 1953). A given response pattern to illumination may be markedly changed by a change in the waking state of the animal. Some units show no response to gross light stimulation during sleep or wakefulness, but do respond to moving objects. As compared with anesthetized preparations, the unanesthetized cat shows much more spontaneous unit activity and a great increase in the variety of responses to stimuli.

274. METABOLIC ADJUSTMENTS TO OXYGEN LACK IN THE PREGNANT UTERUS. William E. Huckabee,* James Metcalfe,* Harry Prystowsky* and D. H. Barron. Boston and Harvard Univs., Boston, Mass.; Johns Hopkins, Baltimore, Md.; and Yale Univ., New Haven, Conn.

Arterio-venous differences in concentration of pyruvate and lactate were studied across the uterus and placenta in normally pregnant, anesthetized goats, and in "hypoxic" animals characterized by low arterial oxygen or low blood pressure. Mean arterial pyruvate was 0.194 mM/l. in normal, and 0.352 in hypoxic animals. Uterine $(A-V)_{pyruvate}$ usually showed "output" by the uterus, and averaged -0.072 mM/l., apparently unrelated to oxygen lack or arterial pyruvate. Fetal levels always exceeded maternal and averaged 0.421 mM/l. in normal, and 0.550 in hypoxic fetuses; no relationship to maternal levels was discernible. Maternal lactate averaged 1.275 mM/l. in normal and 5.944 in hypoxic animals. $(A-V)_{lactate}$ was unrelated to arterial level and showed either output or uptake without regard to oxygen lack. Fetal arterial lactates averaged 2.647 mM/l. in normal, and 10.750 in hypoxic fetuses; fetal concentrations invariably exceeded maternal, but were otherwise unrelated. Maternal lactate-pyruvate ratios averaged 6.89 and 18.08, respectively; corresponding fetal ratios were 6.07 and 25.29. Significantly variable, but random, trans-placental relationships obtained. These concentration values form an orderly system only if it is assumed that: 1) diffusion equilibration across the placenta plays no significant role; 2) the placental $DPNH_2/DPN$ ratio is set by a greater relative oxygen supply than that of fetal or maternal tissues, so that a reduction of calculated "excess lactate" occurs in blood perfusing it; and 3) absolute concentrations then follow from a) adequacy of oxygen supply and b) the lactic dehydrogenase equilibrium. Thus, anaerobic metabolism in hypoxic fetuses provided 60% of their energy requirement; this "oxygen debt," however, was repaid by the placenta and so was not reflected in uterine venous blood.

275. RESPONSES OF ANESTHETIZED DOGS MECHANICALLY VENTILATED AT HIGH FREQUENCIES. Wayland E. Hull. Dept. of Physiology, Duke Univ. School of Medicine, Durham, N. C.

To study physiological events accompanying high transthoracic pressures applied rapidly, a respirator chamber to accommodate a dog was provided with a piston-type pump of 2500 ml displacement, operable at rates up to 18 cps. Transthoracic pressures of 50-60 cm H_2O were produced rhythmically. Oscillographic tracings recorded tidal volume, chamber pressure and intrathoracic pressures from the thoracic esophagus and right atrium. The phase and amplitude of any two events were recorded from x and y axis deflections on a cathode-ray oscilloscope. Averaged data from 9 dogs reveal that above 4 cps, log tidal volume (ml) is a linear function of log frequency (cps) the slope of which is -0.85. The log of the ratio tidal volume (ml)/intrathoracic pressure (cm H_2O) is linear with the log fequency (cps) between 4-18 cps with slope -1.22. Maximum energy absorption, as measured from areas of pressure-volume loops, occurs at 3-5 cps. Phase shift between tidal volume and transpulmonary pressure reaches 90° at 9-11 cps. From these observations, knowing lung volume and rate of change of pressure, prediction may be made of the quantity of gas moved during the decompression or compression phase of the cycle and the level of instantaneous intrathoracic pressure which might produce injury to lung tissue.

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(Supported by Contract 1181(07) Nr 112-416, Office of Naval Research.)

276. KINETICS OF POTASSIUM EXCHANGE IN THE ISOLATED PERFUSED RABBIT HEART. E. W. Humphrey* and John A. Johnson. Physiology Dept., Univ. of Minnesota, Minneapolis.

Two hundred rabbit hearts were perfused with a modified tyrodes solution in a Langendorff preparation in order to study the effect of some variables on the potassium exchange. The rate was varied from 60-200 beats/min at each of two temperatures 20° and 35°. The concentration of potassium in the perfusate was vaired from 2.7-9.0 mEq/l. Using K^{42} as a tracer the hearts, after being perfused with a non-radioactive solution for 30 min, were then perfused with the tracer solution for periods varying from 2-60 min. These studies seem to indicate that the intracellular potassium is not homogenous and exchanges with at least 2 different rates. The effects of the above-mentioned variables on the potassium exchange will be discussed.

277. ENZYME LOCALIZATION IN ERYTHROCYTES. Alice S. Hunter* and F. R. Hunter. Physiology Dept., Southern Illinois Univ., Carbondale.

One aspect of a comparative study of the metabolism of erythrocytes was an attempt to determine where in these cells the glycolytic and Krebs Cycle enzymes were localized. Such an investigation is of interest not only from the standpoint of comparing the metabolism of the non-nucleated mammalian erythrocytes with the nucleated ones of lower vertebrates but also from the more general standpoint of the problem of structural organization of enzymes within cells. Blood was obtained by cardiac or venipuncture and heparin was used as an anticoagulant. The cells were separated by centrifugation and washed 2 or 3 times in the appropriate isotonic saline solution. The red cells were then broken up by alternate freezing and thawing 3 times. With some cell suspensions additional homogenization with a glass homogenizer was necessary to break up the cells satisfactorily. The stroma (including nuclei when they were present) was separated from the remainder of the homogenate by centrifugation at 1000-1500 g. When granules were present in the supernatant fluid, they were separated at 20,000-100,000 g using either an air turbine or a preparative Spinco centrifuge. Manometric assay methods were employed to determine the activity of lactic, malic and succinic dehydrogenase in the various fractions obtained. The data fall into 3 general categories: 1) with mammalian erythrocytes, enzyme activity could be found only in the supernatant fluid; 2) with avian erythrocytes the activity was associated with the stroma-nuclear fraction; and 3) with erythrocytes of lower vertebrates, such as elasmobranchs, the activity was associated with granules.

278. ENTROPIES OF SOME AMINO ACIDS. John O. Hutchens, A. G. Cole,* R. A. Robie* and J. W. Stout.* Dept. of Physiology and Inst. for the Study of Metals, Univ. of Chicago, Chicago, Ill.

As a part of a program to establish thermodynamic constants for the naturally occurring amino acids and their derivatives, the heat capacities of L-glutamine, L-glutamic acid, L-isoleucine, L-methonine, L-phenylalanine, L-tryptophane and L-valine have been measured between 10 and 310°K. The L-glutamine and L-glutamic acid were provided by Alton Meister, the other compounds by Jesse W. Greenstein. All gave acceptable C, H, and N analyses, were free of other amino acids detectable by chromotographic methods, and were free (99.9%) of the D-isomer according to the criteria of Meister et al. (J. Biol. Chem. 192:535, 1951). The method used to measure heat capacities was essentially that described by E. Catalano and J. W. Stout (J. Chem. Phys. 23:1284, 1955). We obtain a precision (maximum deviation of points from a smooth curve) of 1% from 10-20°K, 0.5% from 20-30°K, 0.1% from 30-250°K, and 0.2% above 250°K. As a test of the method, the heat capacity of a Calorimetry Conference Standard sample of benzoic acid obtained from the Bureau of Standards was measured. Essentially we confirm the heat capacities of benzoic acid previously reported (Ginnings and Furukawa, J. Am. Chem. Soc. 75:522, 1953; Osborne et al. ibid. 77:2737, 1955; Busey. ibid. 78:3263, 1956. The values of $S_{298.16}$ and $S_{310.16}$ are: for L-glutamine 46.60 and 48.37; and for L-glutamic acid 44.98 and 46.66. Considering the precision of the heat capacity measurements at various temperatures, the accuracy of the thermodynamic temperature scale, and the likely purity of the compounds these values are probably accurate to 0.2%. The entropies of the remaining substances which offer special difficulties in calculation will be presented. (Supported by Contract No. AT-(11-1)-280 between the Univ. of Chicago and the Atomic Energy Comm.)

279. COORDINATION OF EYE MOVEMENTS IN CATS. Jane E. Hyde and Robert G. Eason.* Dept. of Physiology, Univ. of California Med. Ctr., Los Angeles.

Conjugate eye movements evoked by stimulation of brainstem sites in alert encéphale isolé cats have been observed to be "goal-directed" (Hyde and Eliasson, submitted for publication). Analyzing motion pictures of the eye movements permits calculation of the degree of accuracy of the "goal" for particular sites and situations. Differences in latency, threshold, speed and type of movement are revealed upon stimulation of different brainstem regions from which conjugate eye movements may be evoked. The position of the "goal," with reference to the position of forward gaze, is dependent on the stimulus site. From a single site, in either tectum or tegmentum of midbrain or medulla, the eyes may be induced to move to the same "goal" with an accuracy within a few degrees of the visual field, with intermittent stimulation of the site over a period of up to 2 hr. During this period the eyes may spontaneously have moved to different positions between stimuli. To reach their "goal," different routes must be traversed, so that the direction of the movement may be contralateral, ipsilateral, vertical etc., although the same end point is attained. This implies similar accessibility of the neurons at each conjugate deviation site to all the eye motor nuclei, and suggests that the concept of localized "centers for gaze in different directions" may require some modification when describing eye movements obtained in the alert animal. The "goal" of the eyes for a particular brainstem site is not appreciably altered by either changes in tonic labyrinthine impulses or disorientation of the visual field.

280. DISAPPEARANCE TIME OF UNESTERIFIED FATTY ACID FROM RAT BLOOD. J. M. Iacono, M. I. Grossman, Seymour L. Kirschner, Donald Scheiner and Norman F. Witt (intr. by Thomas H. Allen). U. S. Army Med. Nutrition Lab., Denver, and Dept. of Chemistry, Univ. of Colorado, Boulder.

A complex was prepared containing a 3:1 molar ratio of palmitic acid-1-C^{14} to crystalline bovine serum albumin. The complex was injected into the tail vein of male rats and blood samples were taken

over a 2-hr period following i.v. injection. In addition, expired CO_2 was collected for the 2-hr period. The disappearance of the C^{14} from the blood was so rapid that within 3 min after injection of the complex, only 3-5% of the injected radioactivity could be accounted for in blood. In the 4 groups of animals studied, 4-5 animals per group (fasting control, lipemic, heparinized, lipemic-heparinized), practically no differences were noted in the disappearance of injected fatty acid. Although no differences were noted in rate of clearing of tagged fatty acids from the blood for the 4 groups of animals, it is suggested that the rate of oxidation of the injected fatty acids was different from one group to another. In the course of 2 hr, average percentage recovery of radioactivity in expired CO_2 was 6.4 for the control group; 8.7, lipemic; 11.9, heparinized; 10.9, lipemic-heparinized group. The implications of the above changes in relation to fat metabolism will be discussed.

281. EVIDENCE THAT HISTAMINE IS A GASTRIC SECRETORY HORMONE. A. C. Ivy and Richard W. Schayer. Rheumatic Fever Research Inst., and Dept. of Clin. Science, Univ. of Illinois, Chicago.

One test on each of 6 chronic gastric fistula rats was made in which after a 12-hr fast C^{14} L-histidine was injected subcutaneously. Twenty-four hours later, while still fasting, aminoguanidine HCl (AG) (2 mg of the base), a powerful diamine oxidase inhibitor (an antihistaminase), was given hourly subcutaneously through an indwelling needle day and night for 3 additional days. Twenty-four hours after the start of AG, food was given for 12 hr; fasting then occurred for two 12-hr periods, and then food was given again for 12 hr. The average urinary C^{14} histamine counts/min during the various AG periods were: first AG control period, 105; second AG control period, 104; 12-hr feeding period, 185; first 12-hr postfeeding-fasting period, 157; second postfeeding-fasting period, 68; final 12-hr feeding period, 106. It is concluded that, on feeding, C^{14} histamine was released in quantities adequate to stimulate gastric secretion when aminoguanidine was present to prevent histamine destruction.

282. EVALUATION OF BLUE TETRAZOLIUM REDUCTION METHOD AS A MEASURE OF CORTICOSTEROID EXCRETION. A. J. Izzo, and E. H. Keutmann (intr. by W. Pommerenke). Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Although the chemical reduction of blue tetrazolium (BT) under mild conditions has been shown to be fairly specific for the alpha-ketolic steroids, neutral extracts of urine may contain other substances that reduce BT. It was the purpose of this study to evaluate this method as a measure of the level of urinary excretion of alpha-ketolic steroids in the following manner: 1) to determine the extent to which the non-specific substances reduced BT and how much they contributed to the total reducing capacity of the extract, and 2) to show that a change in the level of excretion of total BT reducing substances in urine accurately reflects a change in excretion of alpha-ketols. Neutral extracts of aliquots of 24-hr urine specimens from 8 normal subjects were prepared by hydrolysis with beta-glucuronidase, continuous extraction with ether for 48 hr, and alkali washing of the crude extract. The total reducing capacity of the neutral extracts was measured by our modification of the BT method (Fed. Proc. 15: 1956). The individual alpha-ketolic steroids in each of the extracts were separated by paper chromatography according to Zaffaroni and Burton and quantitated. All the non-ketol areas of the chromatograms and the overflows were quantitated separately. The sum of all the chromatographic fractions agreed very well with the total reducing value of the original extract in each instance. The alpha-ketols accounted for 80-95% of the total reducing value of the urines studied. The remainder was accounted for by the pigments and substances that gave non-specific ultra-violet absorption, which were found mainly in the least polar fraction of the neutral extracts. While correction could be made in the original extract for those non-ketolic substances that reduce BT in a slow, uniform manner, this did not account completely for the amounts actually found after chromatography. There is indication that some of the non-specific substances may react with BT in a manner similar to the ketols. When 2 human subjects were administered cortisone and hydrocortisone orally, the increase in the total BT reducing value in the urine was accounted for completely by the increase in identifiable ketolic steroids determined by chromatographic analysis. In summary, evidence has been presented to indicate that the total reducing value of a urine extract as measured by blue tetrazolium is a valid although not absolute measure of the alpha-ketol content. However, changes in excretion of total BT reducing substances are an accurate reflection of changes in excretion of adrenal cortical steroids and their reducing metabolites.

283. PLASMA VOLUME CHANGES WITH INCREASED ABDOMINAL PRESSURE. E. T. Jach* and J. P. Marbarger. Aeromedical and Physical Environment Lab., Univ. of Illinois, Chicago.

Male dogs anesthetized with Nembutal were subjected to a sustained abdominal pressure of 10 in. of water introduced into the abdominal cavity through a small pipe extension secured in the abdominal wall. Mean plasma volume (10 dogs) decreased approximately 25% during the first 80 min when compared to pre-existing control values and to anesthetized control dogs (8) with normal abdominal pressure. After 160 min of sustained pressure a recovery trend toward control values was observed. Upon release of abdominal pressure the plasma volume diminished slightly but returned to normal values within 1 hr. During the period of elevated abdominal pressure urine production diminished slightly, but urinary excretion of potassium was markedly decreased when compared to control animals. Serum sodium concentration did not significantly change with sustained pressure, but serum potassium values slightly increased in about one half of the experiments. Mean vena cava pressure at the level of the heart did not markedly change. Mean arterial pressure decreased slightly after pressure was applied, but returned to pre-experimental values before the pressure was released. These observations are compared to similar studies on dogs with increased intrapulmonary pressure.

284. FACTORS WHICH INFLUENCE SURVIVAL OF TRANSFUSED PLATELETS. Dudley P. Jackson, Julius R. Krevans,* Alvin Margolius, Jr.* and Wilbur E. Mattison, Jr.* Dept. of Medicine, Johns Hopkins Univ. and Hosp., Baltimore, Md.

Replacement transfusion of compatible dog blood that had been collected and stored at 4°C in plastic bags containing ACD solution for 7-21 days promptly produced thrombocytopenia in recipient dogs. The maximal depression of platelets was observed immediately after transfusion. Furthermore, autotransfusion of similarly stored blood produced immediate thrombo-

cytopenia. When blood stored for 48 hr was transfused, striking thrombocytopenia occurred but the rate of fall was slower, and the lowest platelet level was observed 24 hr after transfusion. When blood stored for 24 hr was transfused, the thrombocytopenia occurred at an even slower rate and the maximal depression of platelet level occurred 48 hr after transfusion. Autotransfusion of fresh blood did not produce thrombocytopenia. Fresh blood transfusion raised the platelet level in a dog rendered thrombocytopenic by the injection of anti-platelet serum. Except for changes attributable to the thrombocytopenia, studies of blood coagulation gave normal results, including measurements of fibrinogen, fibrinolysis and prothrombin. The effects of glass and silicone-coated glass storage vessels on the survival of transfused platelets were investigated. These experiments demonstrate that platelets contained in fresh blood circulated when transfused. When the blood was stored, the platelets disappeared from the recipient's circulation after various intervals of time. The time of survival of the platelets was inversely related to the time of storage. Survival of transfused platelets could not be correlated with the number of platelets or the clot-accelerating and clot-retracting functions of the platelets in the stored blood.

285. INTESTINAL ABSORPTION OF METHIONINE IN SITU. Francis A. Jacobs and Dale E. Nickerson (intr. by B. DeBoer). Dept. of Biochemistry, Univ. of North Dakota School of Medicine, Grand Forks.

The intestinal absorption of D-, L-, and DL-methionine was studied by an in situ perfusion technique. Adult Sprague-Dawley male rats fasted 20 hr and anesthetized with sodium pentobarbital were employed in all experiments. Solutions of 0.02 M methionine in 0.9% sodium chloride were perfused through an in situ segment of the upper small intestine at a flow rate of 1-2 ml/min. The greatest volume in the perfusion system was 9 ml for an intestinal segment approximately 10 cm long. Samples for analysis were withdrawn from the system at 15-min intervals during a 1-hr perfusion period. It has been reported that glycine exhibited a similar absorption pattern when tonicity of the perfusate was maintained by either 0.9% NaCl or Krebs'-Ringer phosphate buffer (Jacobs et al. Fed. Proc. 15:281, 1956); hence saline was employed in these experiments. Gibson and Weisman (Biochem. J. 48:426, 1951) reported a difference in the uptake for D- and L-methionine, using intestinal segments lower in the intestinal tract. By employing a segment originating 3 cm distal to the pylorus and extending 10 cm distally we have found that the continuous intestinal uptake of D-, L-, and DL-methionine are similar.

286. EFFECT OF FRACTIONATION OF BETA RADIATION UPON RAT SKIN. Elly M. Jacobsen,* A. K. Davis and Edward L. Alpen. U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Female rats were exposed to a $SR\text{-}Y^{90}$ plaque which gives a skin dose of 14.6 r/sec. Four dose fractions of 200, 400, 600, and 1000 r given at either 24- or 48-hr intervals were used to obtain total doses of 3600, 6000 or 12,000 r. A grading and scoring system was devised. Dry desquamation is the maximum effect observed with 200 or 400 r given daily to total doses of 6000 r. Only epilation occurs in animals given 200 r every 48 hr to 12,000 r total dose. For comparison, 3000 r in a single dose produces wet desquamation in 14 days. The threshold for wet desquamation at 600 r/day is 6000 r. At this dose 50% develop the lesion in 19 days after initial exposure. Increasing the total dose to 12,000 r produces 100% incidence. The maximum damage observed at this dose level is not altered significantly by elevating the daily dose to 1000 r. Spacing the dose to 48 hr reduces the incidence of wet desquamation from 50%-0% for the 600 r fraction, 6000 r total dose and from 100%-50% for the 12,000 r total dose. At 1000 r/dose, spacing to 48 hr does not reduce the incidence or severity seen at the 24-hr spacing when equal total doses are compared.

287. NEUROMUSCULAR TENSION STATES IN PERSONS WITHOUT COMPLAINTS: EVALUATION OF ELECTROMYOGRAMS IN HEALTH. Edmund Jacobson. Lab. for Clin. Physiology, Chicago, Ill.

Employing the integrating neurovoltmeter, action-potentials from 4 neuromuscular regions were measured over half-hour test periods in 15 men with platinum-iridium pin electrodes 0.011 in. diameter penetrating muscle and in 15 others with platinum-iridium surface electrodes with dimensions experimentally determined. Four channels were recorded simultaneously, including the right rectus-femoris, the right biceps-brachii, the right massetericus and the left eye region. Mean microvoltage, $\overline{Y} = \int y$ obs. values/n. Percentage of deviations from $\overline{Y}$ were determined for both types of electrodes. Results for $\overline{Y}$ (μv):

	Thigh	Biceps	Jaw	Brow
Surface elect.	0.97±21%	0.84±12%	1.72±30%	4.17±39%
Pin elect.	0.55±25%	1.04±43%	1.42±35%	3.78±29%

The average deviation, $\overline{D} = \int (\overline{y} - y \text{ obs.})/n$, is usually less than 25% of $\overline{Y}$. The trend, m, determined for each individual. $m = \int y - n - b/\int x$, where $b = \int xy \int x - \int y \int x^2/(\int x)^2 - n \int x^2$. In 32% of all muscle group records, m is positive, with pin electrode records predominating 4:1. Values for m range +.225 μv/m to -.240 μv/min and average ±.025. $\overline{V} = \int (y \text{ obs.} - y \text{ cal})/n$ where y cal = mx + b. $\overline{V}$ values are equal to or less than the corresponding values for $\overline{D}$ and represent the variability of tension states aside from the overall change described by the trend m. Generally speaking, surface electrodes proved more sensitive when optimum dimensions were determined.

288. ADRENAL WEIGHT-MAINTAINING ACTIVITY IN PLASMA OF PATIENTS WITH CUSHING'S SYNDROME. Joseph W. Jailer, Donald Longson* and Nicholas P. Christy.* College of Physicians and Surgeons, Columbia Univ., New York City.

A characteristic hyper-response of plasma 17-hydroxycorticosteroids to exogenous ACTH occurred in 14 of 15 patients with Cushing's syndrome due to bilateral adrenal hyperplasia (average post-ACTH level 87 μg %; normal 46; difference in increments significant - Dev/sigma 5.3). The exaggerated response persisted after unilateral adrenalectomy in 6 patients and after suppressive doses of prednisone in 6 others. Previous workers generally failed to demonstrate excess adrenal-ascorbic acid-depleting ACTH in plasma of such patients. In search of an explanation for the hyper-response a study was made of the possibility of another type of corticotropic activity in plasma of subjects with adrenal hyperplasia. Groups of 6-10 rats were injected daily for 9 days starting 1 day after hypophysectomy with 0.9 ml plasma from each of 8 patients. Adrenal weights were compared with uninjected controls. Patients' plasma prevented adrenal atrophy to the extent of 25-60% in 6 of 8 cases (in 4, $P < 0.001$). Sample

differences of mean paired adrenal weights, injected vs. control: 11.9, 7.3 mg; 9.9, 7.6 mg. No adrenal weight-maintaining activity was found in plasma of 1 patient having Cushing's syndrome with carcinoma of the adrenal who lacked hyper-response to ACTH, of normals, or of Addisonian and adrenalectomized subjects. Activity was detected in 3 pools of plasma from women in the third trimester of pregnancy who also exhibited a hyper-response to ACTH similar to that found in Cushing's syndrome.

289. HORMONAL CONTROL OF FAT INFILTRATION INTO LIVERS OF COLD-EXPOSED RATS. R. L. Jasper and H. M. Levy (intr. by L. L. Boyarsky). Dept. of Environmental Medicine, Army Med. Research Lab., Fort Knox, Ky.

Female rats exposed to cold (1-4°C) accumulate fat in the liver at a rapid rate. Liver fat increases 80-90% above the control value after 6 hr cold exposure. This response to cold is not prevented by adrenergic blocking agents. Adrenal demedullation likewise fails to eliminate fatty infiltration of the livers of animals exposed to cold. Bilateral adrenalectomy, however, does affect cold-induced fatty livers. Replacement of adrenocortical hormones does not fully restore the liver fat response of adrenalectomized animals to that of intact female rats when both are exposed to cold. Hypophysectomy of rats eliminates the accumulation of fat in the livers of intact animals exposed to cold. Relatively pure preparations of ACTH do not restore the response of cold-induced fatty livers found in intact animals. Hypophysectomized-adrenalectomized animals respond to cold exposure in much the same manner as animals either hypophysectomized or adrenalectomized. The data indicate an integrated relationship of the pituitary and adrenals. This relationship may not be that conventionally conceived.

290. ABSENCE OF VASOACTIVE SUBSTANCES IN CORONARY SINUS BLOOD OF DOGS. Roger W. Jelliffe,* C. R. Wolf,* Robert M. Berne and Richard W. Eckstein. Depts. of Medicine and Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Indirect evidence indicates that coronary blood flow is largely regulated by alterations in myocardial oxygen tension; vasoactive myocardial metabolites are possibly involved. The question whether vasodilator or vasoconstrictor substances exist in coronary sinus blood has been investigated in dogs. Blood draining the normal, hypoxic and the myocardium perfused at high pressure was oxygenated in a screen-type oxygenator and perfused into a test coronary artery of the same or second animal. Flow rates were compared with those of arterial blood under identical pressures after passage through the same oxygenator. When oxygenated to 91% of control, sinus blood from the normal heart increased test artery flow 10%; from the hypoxic heart, sinus blood oxygenated to 94% of control increased test artery flow 12%; from the over perfused heart, sinus blood oxygenated to 102% of control decreased test artery flow 2%. These alterations of flow are insignificant when compared to the 185% increase in flow into the test artery following release from 15 sec occlusion. The results agree with those obtained by one of us (R.M.B.) who effected oxygenation with a dog lung. It is concluded that either vasoactive materials are not produced by the heart, or fail to enter the venous blood, or are destroyed within the 2 min required for oxygenation. (Supported by Cleveland Area Heart Soc.)

291. ROLE OF ACETYLCHOLINE IN CARDIAC AUTOMATISM. David Jensen (intr. by W. G. Clark). Dept. of Physiological Chemistry, Med. Ctr., Univ. of California and VA Ctr., Los Angeles.

The purpose of these investigations has been to examine in several species the hypothesis advanced by Burn and his associates (Lancet 1161, June 1953), that the acetylcholine-cholinesterase system may stimulate as well as inhibit myocardial muscle and thus play a role in cardiac automatism, arrythmias and fibrillation. Cardiac tissue from lamellibranch molluscs (5 species), cyclostomes (2 species), amphibia (2 species) and mammals (2 species) were studied. Isolated heart muscle was tested under the following limiting conditions of spontaneous activity, to determine whether or not acetylcholine exerted a stimulating effect: cold, anoxic anoxia, nitrogen anaerobiosis, cocaine, atropine, spontaneous hypodynamia or application of proguanil (paludrine). Drug concentrations are expressed as gm/ml of the salts. Lamellibranch hearts were only inhibited by acetylcholine, 10^{-4}-10^{-12}, no stimulation was observed. Similar results were obtained with amphibian tissue. In the lamprey heart, marked stimulation occurred with acetylcholine 10^{-6}, whereas the nerveless hagfish heart was totally refractory to acetylcholine 10^{-2}-10^{-6}. The rat and rabbit auricle were sharply stimulated by acetylcholine, provided that the isolated tissue was maintained carefully and was hypodynamic. Once acetylcholine restored automatism in these auricles, however, subsequent applications were always inhibitory. It has been concluded from these studies that the acetylcholine-cholinesterase system does not play a role in cardiac automatism of all species. (Supported by an Established Investigatorship, Riverside Ct. Heart Assoc., and grants from the Los Angeles Ct. Heart Assoc.)

292. CORRELATION BETWEEN PRESSURE AND FLOW CHANGES IN THE UNANESTHETIZED DOG. R. Jensen, A. Kolin, N. S. Assali and G. Herrold (intr. by John Field). Univ. of California, Los Angeles.

The blood flow was measured by means of a miniature implanted electromagnetic flowmeter (Kolin. Proc. Soc. Exp. Biol. & Med. 35:53, 1936; 46:235, 1941). The transducer consisting of a small a.c. electromagnet embedded in a non-irritant plastic, together with pick-up electrodes, contains a round channel into which the intact artery fits closely. The magnet is energized by a 60 cycle a.c. The instrumentation is the same as described in an earlier paper (Kolin. Revise. Inst. 23:235, 1952). A polyvinyl occlusion bag is placed around the artery to secure a zero reading at zero flow. The flowmeter and the occlusion bag are implanted under sterile conditions and the measurements are performed after the animal has recovered from anesthesia. The arterial pressure is recorded by inserting a polyvinyl tubing intra-arterially during the same operation at a point proximal to the flowmeter. This tube is permitted to emerge through the skin and is attached to a pressure transducer for recording. The leads for current supply to the magnet as well as the pick-up leads are encased in polyvinyl tubing which is exteriorized through the skin. Simultaneous records of arterial blood flow, arterial blood pressure and respiration were taken. Effects of various physiological and psychological stimuli and pharmacological agents were observed. Comparisons were made between observation in anesthetized and

nonanesthetized dogs with special reference to the pressure-flow characteristics. (Supported by grants from the Office of Naval Research, Los Angeles Heart Assoc. and USPHS (Grant 1755 C3).)

293. TIME-RELATIONS BETWEEN MUSCULAR CONTRACTION AND RESPONSE OF CYTOCHROME CHAIN. Frans F. Jöbsis* and Britton Chance. Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.

Other work (A. Weber, Fed. Proc., this issue) had shown that the respiratory chain of excised frog muscle responds to twitching as isolated sarcosomes do to the addition of ADP in the presence of substrate. The present work first, verifies these findings for a variety of other vertebrate skeletal muscles and, secondly, compares the kinetics of the changes of the steady-state oxidation-reduction levels with the isometric myogram. Controls on non-specific light scattering, absorption and diffraction changes were made in collaboration with W. Sleator, Jr., and a muscle holder of a design suitable for measuring rapid spectroscopic changes and for simultaneous recording of mechanical changes was constructed. Results with this device show, for the turtle coracohyoideus, that the initiation of the response of the cytochromes is delayed until the twitch tension has gone to approximately 80% of completion (~ 100 msec at 20°C) and that even greater asynchrony exists in the recovery phases. This lag varies among different muscles (frog and toad sartorius and turtle retractor penis) but is always present. Calculations of the ADP diffusion time from unfavorable locations are at least 5 and probably 11 times shorter than the observed lag. It is concluded that no free ADP is released by the muscle contraction until the tension development is largely completed.

294. EFFECTS OF INTRAVENTRICULAR INJECTIONS ON CONDITIONED RESPONSES. E. R. John,* K. L. Chow,* M. Gatsch,* B. Wenzel* and R. D. Tschirgi. Dept. of Physiology, Univ. of Calif. Med. Ctr., Los Angeles. Cannulae were implated

Cannulae were implanted into the lateral cerebral ventricles of cats, enabling direct injection of solutions into the cerebrospinal fluid without anesthesia. These animals were subsequently conditioned to avoidance responses to tone and light, in a double grill box, and to pattern discriminations for food, in a Yerkes box. The effects of central injection of a number of materials were studied, using as indices the average latency of avoidance responses in a set of trials, the number of avoidance responses versus the number of escape responses or the number of undirected responses in a set of trials, the percentage of correct pattern discriminations in a set of trials, and the appearance of position habits during a set of trials of pattern discrimination. It was found that reserpine had characteristic effects on avoidance behavior, with reversible increases in latency occurring as a function of time after injection. As dosages increased, a family of latency curves was generated. At sufficiently high doses, avoidance responses became replaced by escape responses and finally by undirected responses. The interaction of centrally injected epinephrine, norepinephrine, serotonin and iproniazid with these reserpine effects was studied. In addition, the effects of eserine, sodium azide, potassium chloride and calcium chloride injections were studied using the above indices.

295. EFFECT OF RISING ENVIRONMENTAL TEMPERATURE ON THE THYROID I^{131} ACTIVITY. H. D. Johnson, C. S. Cheng and Samuel Brody[1] (intr. by H. E. Dale). Dept. of Dairy Husbandry, College of Agriculture, Univ. of Missouri, Columbia.

Rising environmental temperature (48°-110°F) depressed the in vivo release rate of hormone I^{131} from thyroid gland of rabbits. Thyroid activity of both male and female rabbits declined from approximately 14 empirical units at 48°F to 1.5 at 105°F. Does environmental temperature and/or body temperature directly (neural or humoral) depress the thyroid via the hypothalamus? Or is the thyroid activity depressed by the lessened utilization of the blood thyroxine (thus inhibiting the pituitary) due to the animals lowered feed consumption and heat production? Associated with declines in thyroid activity are decreases in feed consumption and heat production and rise in rectal temperature at 80°F. Feed consumption declined from 125 gm at 80°F to 3 gm at 110°F. Heat production decreased from 20 cal/hr at 80°F to 15 cal/hr at 100°F. Rectal temperature increased at 80°F and attained 107°F at 105°F. Ratios of thyroid activity/metabolism and feed/metabolism indicated a decrease from 0.5 at 80°F to 0.1 at 100°F. Ratio of feed/thyroid remained somewhat constant from 48° to 105°F indicating similar relative changes. These data point to a close relationship of feed consumption and thyroid activity and an inverse relationship of these functions to body temperature—even indicating that possibly the feed consumption level (via appetite center) at these higher environmental temperatures may control the thyroid activity. (Supported by Atomic Energy Comm.)

296. COAGULATION COMPONENTS FOLLOWING INTRAVENOUS ESTROGENS. J. Frederic Johnson (intr. by F. Gaynor Evans). Dept. of Physiology and Pharmacology, Wayne State Univ., Detroit, Mich.

Reports have indicated that an advantageous therapeutic result can be obtained in the treatment of hemorrhage from the upper respiratory tract, as well as from other areas, by using intravenous estrogens. This result is often obtained after other measures have failed. A laboratory investigation of possible alterations of certain plasma coagulation factors accompanying the administration of an estrogenic substance was undertaken. Concurrent with intravenous use of estrogens in the dog and human there is a sharp, rapid rise in the prothrombin and acceierator globulin content of the plasma as well as a fall in the antithrombin concentration. Platelet cofactor I, autoprothrombin I and II, and fibrinogen have been determined during the same period. Slight alterations in their plasma levels have been noted. These changes of the clotting components coincide with the observed period of clinical usefulness and would tend to enhance the coagulation of the blood. In our integration, the physiology of blood coagulation is connected with hormone function.

297. PROTHROMBIN AND ITS DERIVATIVES IN RELATION TO ANTICOAGULANT THERAPY. Shirley A. Johnson. Dept. of Labs., Henry Ford Hosp., Detroit, Mich.

Several reports in the past 2 yr have stated that autoprothrombin II, as well as prothrombin and autoprothrombin I (factor VII, co-thromboplastin), is decreased by the administration of oral anticoagulants, Dicumarol, Sintrom, Hedulin and Dipaxin. The changes in autoprothrombin II do not influence the Quick prothrombin time. Therefore, these changes are not

[1]Deceased Aug. 6, 1956.

reflected in the laboratory test usually used to follow anticoagulant dosage. Clinical observation has indicated that Marcumar is an excellent anticoagulant for long-term management. The ease of control with this drug, using the Quick prothrombin time, suggests that all the factors affected by the drug influence this test. Prothrombin and autoprothrombin I are reduced drastically, the latter first, of course, and remain reduced as long as the drug is administered. The levels of autoprothrombin II in the serum remained unchanged, however, even for several years of drug administration. The plasma and serum levels of autoprothrombin II of these patients varied considerably, but were all within the range of the 35 normals reported by Johnson, Seegers, Koppel and Olwin. The autoprothrombin I values were always higher in serum due probably to either a conversion of prothrombin to autoprothrombin I when the blood clotted or to a release of platelet co-thromboplastin liberated on disintegration of the platelets.

298. DIFFUSION OF CO_2 ACROSS THE RENAL TUBULE. John T. Kaim,* Gaspar Carrasquer,* Robert Shapiro* and William A. Brodsky. Inst. for Med. Research, Univ. of Louisville School of Medicine, Louisville, Ky.

A classical dictum holds that CO_2 penetrates all biological membranes with great ease. Therefore, differences of CO_2 tension between urine and plasma have been explained by the following processes: hydrogen ion secretion, bicarbonate ion reabsorption, delayed dehydration or delayed hydration of CO_2, back-diffusion of lactic acid, or mixing of fluids of different pH from various nephrons. Experiments were designed to test the validity of the aforementioned postulates. Anesthetized dogs under conditions of mannitol diuresis were given inhalations of 20% CO_2-80% O_2. The expected decreases of blood and urinary pH were found after CO_2 inhalation. However, the pCO_2 of pelvic urine increased slowly, remaining less than that of renal venous plasma for 30-60 min. Intravenous injection of carbonic anhydrase produced a great increase in carbonic anhydrase activity of the urine, but failed to obliterate the large CO_2 tension difference between urine and renal vein plasma. Such data imply: 1) that a partial barrier to CO_2 diffusion must be present in the renal tubule; 2) that urinary acidification during CO_2 inhalation can be explained by reabsorptive transport of bicarbonate ion rather than by secretion of hydrogen ions. The latter process would tend to make urine pCO_2 greater than, or equal to plasma pCO_2—i.e. exactly opposite to that observed. (Supported by USPHS Grant A-461, and by U. S. Army Contract No. DA-49-007-MD-481.)

299. RESPONSE OF MOUSE SKIN TO HORMONES AND UNSATURATED LIPIDS. A. A. Kandutsch (intr. by J. L. Fuller). Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine.

The amounts of Δ 7-cholestenol and cholesterol in the skins of mice are altered independently of each other by the application of carcinogenic hydrocarbons, hyperplastic agents or by castration. Cholesterol is present in epidermal cells and in the dermis, while Δ 7-cholestenol is concentrated in the sebaceous glands. Localization of these sterols in separate morphological components of the skin suggests that a study of sterol changes might contribute to an understanding of the physiology of this organ. Male and female mice, normal and castrated, were treated with hormones or unsaturated lipids either by injection or by topical application. Changes in the weight of epidermis, dermis, Δ 7-cholestenol and cholesterol/unit area of skin and of cholesterol, Δ 7-cholestenol and fat per gram of dry fat-free tissue were determined. Treatment with testosterone or progesterone or with estrogen in oil caused a marked increase in the amount of Δ 7-cholestenol accompanied by small changes in the amount of cholesterol and epidermal and dermal weight. Treatment with cortisone caused similar minor changes; adrenalectomy had no apparent effect. The application of squalene increased the amount of Δ 7-cholestenol as much as 130% but had little effect on cholesterol and epidermal weights.

300. EFFECT OF HEPATECTOMY UPON LIPID MOBILIZATION. Alex Kaplan and Stafford Jacques.* Dept. of Biochemistry, Med. Research Inst., Michael Reese Hosp., Chicago, Ill.

The preparation by Seifter and Baeder (Proc. Soc. Exp. Biol. & Med. 91:42, 1956) of a plasma fraction which has potent lipid-mobilizing effects provided us with a tool for studying the role of the liver in fat mobilization. Active extracts of Lipid Mobilizer (Wyeth-LM991) were injected intravenously into a) fasted, intact Holtzman rats and b) fasted rats that were eviscerated and hepatectomized; c) 0.9% NaCl solution was injected into another group of eviscerated-hepatectomized rats. The volume of solution in all groups was 0.1 ml/100 gm body wt. or 5 mg/kg of Lipid Mobilizer. Approximately 0.4 ml of blood was taken from the tail for lipid analysis prior to the injection of LM extract or NaCl. The animals were sacrificed 1 hr postinjection and blood was removed from the vena cava. The Lipid Mobilizer produced a visible hyperlipemia in the intact rats, with a rise of 90-300% in the concentration of serum triglycerides; the alterations in the levels of phospholipid and cholesterol, however, were minor. In contrast to this, the concentration of triglycerides decreased by approximately 50% and phospholipid and cholesterol by about 25% in both of the eviscerated-hepatectomized groups, irrespective of the type of injection. The administration of Lipid Mobilizer failed to alter the characteristic fall in the concentration of serum lipids that follows evisceration-hepatectomy. It appears that the liver and/or viscera are essential for the Lipid Mobilizer to produce a hyperlipemia. (Aided by a grant from the American Heart Assoc.)

301. ANESTHESIA IN TURTLES. Harold M. Kaplan and Richard Taylor.* Dept. of Physiology, Southern Illinois Univ., Carbondale.

Ether, Nembutal and urethane effectively anesthetized turtles. The effect upon heart rates was obtained by surface electrocardiograms or by observing the exposed pericardium. Rectal temperatures were followed with a deeply placed thermometer. Ether in about 30 min induced deep anesthesia persisting at least 3 hr, involving early muscle relaxation, absence of gross movements and eventually lowered heart rate and rectal temperature. Nembutal was effective intraperitoneally (i.p.), intravenously (i.v.), and into the heart (i.c.) by retrograde cervical approach, in average doses of 16.3, 17.6 and 15.5 mg/kg, respectively. In 30 min-1 hr it induced deep anesthesia, i.c. being quickest and i.p. slowest. The effect persisted several hours, i.p. being most prolonged. Muscles were usually relaxed in 1 hr although relaxation was promoted earlier and more often with ether. Rectal temperatures eventually fell; heart rates remained high. Urethane was least satisfactory, averaging 126 to 270 min, variable with route, to induce deep anesthesia; most animals were unconscious at least 10 hr. However, it produced good muscle relaxation in about 2 hr, especially with i.v. and i.c.

injections. This persisted several hours. Gross movements lessened or disappeared. Urethane was generally effective by oral, i.p., i.v. and i.c. routes, in average doses of 2730, 2780, 2380 and 1720 mg/kg, respectively.

302. EFFECT OF FARNESOL AND SQUALENE ON CHOLESTEROL SYNTHESIS IN RAT LIVER. Esko Karvinen and Perttu V. Laakso (intr. by D. Nelson). Dept. of Clin. Science, Univ. of Illinois, and Research Dept. Bauer and Black Co., Chicago.

Harlan male rats were fed a cholesterol-free basal diet (BD) for 3 wk. Isocaloric rations were administered to each rat every 48 hr. Group I (5 rats) received BD only. Group II (5 rats) received BD + 0.3 cc of squalene mixed with BD every 48 hr. Group III (5 rats) received BD + 0.3 cc/48 hr of farnesol mixed with BD. Twenty hours after administering the last ration all rats had finished their food. The animals were then fasted another 28 hr. After these 28 hr, acetate-1-C^{14} in saline was injected i.p. and 75 min after the injection the rats were killed. Total liver sterol was isolated as sterol digitonide, washed and counted in a Geiger counter. Fecal excretion of ether soluble material was determined and it indicated that the absorption of farnesol was almost complete, whereas only a small fraction of squalene was absorbed. The total liver sterol was found to amount to 15, 33 and 16 mg/liver in Group I, II and III respectively. The mean specific activity of liver sterol was 1.9, 0.08 and 1.8 cpm/mg sterol in Group I, II and III, respectively. These results show that squalene feeding led to an accumulation of sterol in the liver and prevented the uptake of acetate carbon in liver sterol, whereas farnesol feeding had neither of these effects.

303. ANTICHOLINESTERASES AFFECT TRANSMEMBRANE POTENTIALS AND MECHANICAL ACTIVITY OF RABBIT AURICLES. Seymour Katsh and Jean M. Marshall. Dept. of Embryology, Carnegie Inst. of Washington, and Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, Md.

Auricles were maintained at 30±0.5°C in modified, oxygenated Kreb's solution (pH 7.4). A KCl-filled microelectrode and an RCA transducer no. 5734 were used to record potentials and contractions respectively. All compounds were dissolved in the Kreb's solution and brought to pH 7.4. The following observations on pacemaker and non-pacemaker tissue were noted. Mecholyl (10^{-5}-10^{-4} M) slightly increased the height of membrane and action potentials while inducing a striking decrease in duration of action potential, rate and tension: between 10^{-4} and 10^{-3} M, Mecholyl obliterated electrical and mechanical activity. Carcholin (10^{-5}-10^{-4} M) decreased the duration of the action potential, rate and tension; between 10^{-4} and 10^{-2} M, Carcholin arrested all activity. Neostigmine (10^{-5}-10^{-2} M) elicited variable responses: initially inhibition of electrical and mechanical activity was observed; repeated exposure led to a stimulation of rate and tension. Inhibition or arrest following any drug was overcome by atropine (10^{-3} M) and by epinephrine (10^{-3} M). Reactivation by epinephrine was accompanied by marked increase in rate and height of contraction.

304. DIURESIS IN THE X-IRRADIATED RAT. Robert E. Kay and Cecil Entenman (intr. by I. L. Chaikoff). U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Edelmann (Nucleonics 8:28, 1951) noted a marked polyuria on the first postirradiation day in intact but not in hypophysectomized rats. On the other hand, our observation that a diuresis is produced in head-shielded, body-irradiated rats but not in head-irradiated rats suggests that the effects are not due to direct action of the x-rays on the pituitary gland. Techniques of partial-body irradiation and irradiation of organectomized rats were used to find the organ or body area which must be irradiated before diuresis occurs. Sprague-Dawley female rats weighing 190-210 gm were fasted for 24 hr prior to and after exposure to 600 r of 250 KVP x-rays and urine collections were made on the day after irradiation. Irradiation of the head, legs or exteriorized gut did not cause diuresis and shielding of the head, legs or exteriorized gut did not prevent it. In addition adrenalectomy or splenectomy did not prevent diuresis in the x-irradiated rat. Thus it appears that the adrenals, spleen, head, legs and gut are not factors in the x-ray induced diuresis of the rat. On the other hand, although diuresis did not occur upon x-irradiation of exteriorized kidneys, shielding of the exteriorized kidney prevented diuresis. Thus it appears that the kidneys must be irradiated before diuresis will occur, but irradiation of the kidneys themselves does not cause diuresis. Therefore, in addition to the presence of the pituitary gland and irradiation of the kidneys a third condition is needed to cause diuresis. Irradiation of the pancreas may be this third condition since diuresis does not occur in fasted whole-body x-irradiated, pancreatectomized rats. The relation of the pancreas, pituitary and kidneys to the x-ray induced diuresis in the rat will be discussed.

305. EFFECT OF PHLORIZIN ON OXIDATIVE METABOLISM IN KIDNEY. Daniel M. Keller* and William D. Lotspeich. Dept. of Physiology, College of Medicine, Univ. of Cincinnati, Ohio.

Recent studies in this laboratory (J. Biol. Chem. 222:843, 1956) have shown that phlorizin inhibits oxidative metabolism in kidney in vitro by depressing both oxidation and phosphorylation. Recent experiments have sharply differentiated this effect from classical uncoupling of oxidation and phosphorylation. In homogenates oxidizing citrate, 2,4 dinitrophenol caused typical uncoupling. However, phlorizin still depressed respiration and adenosine phosphates still reversed this effect. Further studies have been done using kidney mitochondria; α-ketoglutarate was substrate, ADP was added, malonic acid was present to block succinic dehydrogenase and glucose-hexokinase system was present as phosphate acceptor. Good P/O ratios of 2.40 were achieved. In this system, phlorizin, 5 x 10^{-4} M, depressed P/O ratios to 1.20. Moreover, the ability of phlorizin to do this depended strikingly on the steady state of the respiratory components in the mitochondria at the time phlorizin was added. Mitochondria were first preincubated with phlorizin before addition of α-ketoglutarate. Here at time of phlorizin addition adenosine diphosphate in the mitochondria remained as such and electron carrier coenzymes were oxidized. Under these circumstances when α-ketoglutarate was added respiration was markedly depressed (approximately 52%). In contrast, however, when mitochondria were preincubated with α-ketoglutarate before addition of phlorizin, the phlorizin was essentially without effect. Here adenosine diphosphate had been converted to ATP and electron carrier coenzymes were partially reduced. These findings are discussed in relation to kinetics and mechanism of action of phlorizin.

306. RESPIRATORY RESPONSES TO ACUTE CHANGES IN O_2 AND CO_2 DURING ACCLIMATI-

ZATION TO HIGH ALTITUDE. R. H. Kellogg, B. E. Vaughan* and D. W. Badger.* Dept. of Physiology, Univ. of California, Berkeley, and White Mountain Research Station, Big Pine, Calif.

To investigate the relative importance of O_2 and CO_2 in regulating pulmonary ventilation during acclimatization to high altitude, 4 adult male sea-level residents were studied at Berkeley (250 ft) and then flown to the White Mountain Research Station for additional study during 2 or more weeks' residence at the Summit Laboratory (14,250 ft). To test chemoreceptor responsiveness, respiration was measured while subjects breathed CO_2-free gas, the O_2 content of which was initially high but subsequently lowered so that alveolar oxygen ($P_{A_{O_2}}$) fell slowly from 225-45 mm Hg. As acclimatization progressed, ventilation in these tests was increased by 3-6 l./min at all levels of $P_{A_{O_2}}$, without change in hypoxic threshold. To test CO_2 response, ventilation was measured while inspired CO_2 was slowly increased and O_2 was adjusted to keep $P_{A_{O_2}}$ constant at 225, 100 or 55 mm Hg during the test. The $P_{A_{CO_2}}$ required to produce any given level of respiratory stimulation decreased about 13 mm Hg during the first few days of acclimatization, and regardless of this large readjustment, was always about 1-3 mm Hg lower when $P_{A_{O_2}}$ was fixed during the test below the hypoxic threshold (55 mm Hg) than when it was fixed above the hypoxic threshold (100 or 225 mm Hg). These results indicate that chemoreceptor responsiveness to hypoxia remains essentially constant during respiratory acclimatization to this altitude, and not merely initiates the respiratory adjustments but also continues to contribute a small but significant stimulus to respiration throughout the large readjustments in CO_2 response. (Supported by Office of Naval Research contract.)

307. ACCELERATION OF HEPATIC CHOLESTEROL SYNTHESIS IN DOGS BY BILIARY FISTULAE. F. B. Kelly, Jr.,* Stephen G. Economou,* M. Stumpe,* C. B. Taylor and G. E. Cox.* Dept. of Pathology, Presbyterian-St. Luke's Hosp., Chicago, Ill.

An in vitro incubation technique has been used to estimate the rate of cholesterol synthesis, from 1-C14-sodium acetate, in viable liver biopsies surgically removed from dogs with altered biliary physiology. Studies have included biliary stasis (common duct ligation), biliary fistulae (cholecystonephrostomy) and dietary supplementation with bile and bile acids. When possible, serial studies were done on individual animals to permit each animal to serve as its own control. Synthesis rates were calculated in units of milligrams of newly synthesized cholesterol per 100 gm of liver/hr. Hepatic cholesterol synthesis rates in 21 control dogs ranged from 0.2 to 1.2 (average: 0.65) mg/100 gm/hr. In 6 animals with biliary stasis of 2-wk duration, synthesis rates ranged from 1.1-3.3 (average: 2.4); individual animals showed an average elevation of 4 times their respective control rates. In 8 dogs with biliary fistulae of 3 or more wks duration, synthesis rates ranged from 1.2-3.3 (average: 2.1); individual animals showed an average elevation of 3 times their respective control rates. Two animals with fistulae of 4 and 8 months duration, showed less elevation of synthesis rates terminally than initially. Preliminary studies have shown no significant effect of daily feeding of physiologic amounts of canine gallbladder bile or cholic acid to control and fistula animals. (Supported by Chicago and American Heart Assocs., Life Insurance Med. Research Fund and Otho S. A. Sprague Mem. Inst.)

308. INTRAVENTRICULAR PRESSURE RESPONSES TO CARDIAC NERVE STIMULATION. Albert Kelso* and Walter C. Randall. Dept. of Physiology, Stritch School of Medicine and the Grad. School, Loyola Univ., Chicago, Ill.

Several previous reports from this laboratory have emphasized the differentiation of augmentor from accelerator cardiac responses to electrical stimulation of the cardiac sympathetic nerves. Prompt and sustained elevation of systolic pressure with little or no change in diastolic pressure indicates that increased systolic ejection is chiefly responsible for augmented myocardial contraction. The present experiments were designed to study the dynamic mechanisms concerned. Pressures were simultaneously recorded from the carotid artery and the right and left ventricles, together with cardiometer tracings during electrical excitation of the stellate ganglia. Ventricular end-diastolic pressures generally decreased slightly while systolic pressures increased, thus contributing to a significant elevation in arterial pulse pressures and greater emptying of the ventricles. Both the time of onset and the intensity of response of the 2 ventricles were somewhat dependent upon which of the stellate ganglia were stimulated. Upon occlusion of the azygos vein, superior and inferior venae cavae, pressures in the right ventricle promptly declined while similar changes in the left ventricle were considerably delayed. Upon release of occlusion right ventricular pressures rose promptly while similar elevations in left heart pressure were delayed some 6-10 cardiac cycles. These studies emphasize the importance of the pulmonary vascular reservoir in supplying the blood required for sustained elevation of left ventricular output.

309. RESPONSES OF A CRUSTACEAN PHOTORECEPTOR. Donald Kennedy (intr. by V. F. Lindeman). Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.

The present experiments are concerned with the properties of a photoreceptor, first described by Prosser (J. Cell. & Comp. Physiol. 4:1934), which is located in the 6th abdominal ganglion of the crayfish ventral nerve cord. Whole cords were excised and stored in cold Van Harreveld's solution. Gross-electrode records of impulse activity in such cords indicate that only 4 or 5 fibers carry the photosensory discharge; the entire cord can thus be used as a few-fiber preparation. At low stimulus intensities, a smooth function of impulse frequency vs. time is found; first-spike latency may be as high at 3 sec, and adaptation is very slow. At high intensities, a "fast" frequency peak appears (latency 200-300 msec), usually followed by a postexcitatory depression and then a smaller secondary increase. Stimulus intensity and duration are apparently reciprocal up to at least 1 sec duration if impulse frequency is used as the criterion; but latency is shorter in the response to the shorter, more intense flash, suggesting that separate processes determine frequency and latency. Some 6th ganglia show a small area of red pigment. Microspectrophotometric measurements of its absorption spectrum agree with data on spectral sensitivity of the response; both have maxima near 500 mμ. The pigment is apparently contained in neurone cell bodies and axons within the ganglion. This pigment, while spectrally similar to the vertebrate rod pigment rhodopsin, does not visibly bleach upon illumination. The receptor nevertheless undergoes a reversible sensitivity change upon light adaptation.

XUM

310. INTERPRETATION OF URINE CO_2 TENSION. Thomas J. Kennedy, Jr., Murray Eden* and Robert W. Berliner. Natl. Heart Inst., Bethesda, Md.

The effect of buffer on CO_2 evolution from bicarbonate solutions after addition of strong acid was studied. Buffer delays attainment of equilibrium in direct relation to buffer concentration and initial bicarbonate concentration. In the absence of buffer, added acid is converted almost quantitatively to carbonic acid and the rate of formation of CO_2 is proportional to the carbonic acid yet to be dehydrated. When buffer is present added acid titrates buffer as well as bicarbonate and, although in the attainment of equilibrium virtually the same amount of CO_2 must be formed, the concentration of carbonic acid driving the reaction is only a fraction of that in the unbuffered system. If CO_2 dissipation is prevented before attainment of equilibrium the CO_2 tension rises as the system proceeds to equilibrium. These data bear on the interpretation of experiments relating the difference between plasma and urine CO_2 tension to buffer concentration of urine. Assuming no diffusion limitation, a mechanism similar to that described is adequate to explain most of the experimental observations in the dog, assuming that tubule urine reaches a segment relatively impermeable to CO_2 before attainment of equilibrium because of the presence of buffer. The rapidity with which equilibrium is reached in unbuffered bicarbonate solutions makes it unlikely that significant CO_2 tension elevation would be found in unbuffered urine. This reinterpretation, while compatible with the mixing hypothesis previously proposed, makes it unnecessary except as a possible explanation for urine CO_2 tensions below that of plasma.

311. GLUCOSE TITRATIONS IN ACUTE ANEMIA. R. H. Kessler,* O. P. A. Heidenreich* and R. F. Pitts. Dept. of Physiology, Cornell Univ. Med. College, New York City.

When the plasma glucose concentration of the normal dog is progressively increased, tubular reabsorption is complete until the filtered load exceeds Tm glucose. A plot of the titration curve exhibits essentially no splay. This implies that filtration capacities of individual glomeruli are precisely balanced by the reabsorptive capacities of their attached tubules. According to Pappenheimer's and Kinter's thesis of autoregulation of function, plasma is skimmed at the origins of the afferent arterioles from the interlobular arteries. Centrally placed glomeruli receive predominantly plasma; those periferally placed receive predominantly red cells. This view implies that the central glomeruli, producing more filtrate, are attached to tubules of greater reabsorptive capacity, whereas peripheral glomeruli, producing less filtrate are attached to tubules of lesser reabsorptive capacity. Pappenheimer and Kinter maintain that reduction of hematocrit has little effect on the filtering capacity and resistance to flow of central glomeruli but does increase filtration rate and reduce resistance to flow of more peripheral ones. Were this true, the glucose titration curve in anemia should exhibit increased splay, for now the peripheral nephrons, filtering more, would reach their individual Tm values at lower plasma levels. In the present study, glucose titrations were performed in dogs with normal hematocrits and with hemotocrits of 4-10%. Results show no significant difference in splay between the normal and the acutely anemic dogs.

312. CHANGES IN GASTRIC MOTILITY OF THE RAT DURING RADIATION EXPOSURE. Donald J. Kimeldorf. Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Gastric motility during radiation exposure was studied in the intact rat by means of an intragastric balloon and water manometer technique using an ink-writing kymographic recorder. Animals were lightly anesthetized with Dial in Urethane and tracheal catheterization was performed under supplemental ether anesthesia. A 2-cm diam. spherical balloon, connected by means of a stiff catheter (2.2 mm o.d.) to the manometer, was passed down the esophagus, located in the stomach, and inflated to 10 cm pressure (H_2O). Three physiological states of gastric motility in the anesthetized animal were studied with respect to radiation exposure: 1) in the untreated animal, 2) in animals where motility was augmented by a central acting drug (reserpine) and 3) in animals where motility was augmented by a peripheral acting drug (pilocarpine). After a regular pattern of motility had been obtained the animal was exposed to 85 kv or 250 kv x-rays at various dose rates. The effect of x-irradiation was readily apparent and similar in all 3 conditions of motility. After an initial latency there was a depression in motility which was manifested in the amplitude rather than in the frequency of contractions. The change in motility was evident within 5-9 min after the start of exposure and lasted for a duration which was related to the exposure dose. The minimum effective dose was approximately 1000 r (air). The depression of gastric motility during radiation exposure appears to be considerably less radiosensitive than other gastrointestinal responses such as increased food or dye retention which occur at a comparable time.

313. EFFECT OF INSULIN ON ETHANOL METABOLISM IN DIABETIC DOGS. F. W. Kinard, E. C. Cox* and M. G. Hay.* Physiology Dept., Med. College of South Carolina, Charleston.

The rate of alcohol metabolism was determined in fasted dogs following intravenous administration of ethanol, in doses of 2.0 gm/kg. The rates were determined with and without insulin on different days. After control rates had been established, diabetes was induced with alloxan. The rate of alcohol metabolism, with and without insulin administration, was restudied. The rate of ethanol metabolism was increased in both normal and diabetic animals 13.7 and 7.3%, respectively, following subcutaneous injection of regular insulin, in doses of 1 U/kg. It appears that the rate of alcohol metabolism is somewhat accelerated in both groups of animals, following administration of insulin. (Supported in part by a grant from the Committee on Problems of Alcohol, Natl. Academy of Sciences–National Research Council.)

314. EFFECT OF EPINEPHRINE ON CARBOHYDRATE METABOLISM OF CO_2 NARCOTIZED RATS. C. T. G. King (intr. by C. M. Williams). U. S. Naval Med. Research Lab., U. S. Naval Submarine Base, New London, Conn.

Normal and adrenalectomized mature male rats of the Wistar-Hisaw strain were narcotized by exposing them to an atmosphere of 30% carbon dioxide in oxygen for periods of up to 5 hr. Epinephrine hydrochloride was injected s.c. just prior to exposure at a dose level of .02 mg/100 gm of body weight. Liver, muscle glycogen and blood sugar determinations were carried out 1, 2, 3, 4 and 5 hr after injection. In the normal narcotized rat epinephrine affects neither the muscle glycogen stores nor the carbon dioxide produced hyperglycemia but prevents the "cyclic"

re-accumulation of liver glycogen observed under conditions of carbon dioxide narcosis alone. In the adrenalectomized rat epinephrine does not change the lowering of liver glycogen produced by high concentrations of carbon dioxide but reverses the previously observed hypoglycemia to hyperglycemia at the apparent further expense of glycogen stores in the muscle which are practically depleted. These studies indicate that epinephrine inhibits the re-accumulation of liver glycogen which occurs in rats under conditions of carbon dioxide narcosis.

315. RENAL TUBULAR REABSORPTION AND SECRETION OF DIODRAST IN NECTURUS. William B. Kinter (intr. by J. R. Pappenheimer). Dept. of Physiology, Harvard Med. School, Boston, Mass.

Although diodrast and related substances are known to be secreted by renal tubules of all vertebrates heretofore studied, no published data for Necturus could be found. In the present study renal plasma clearances of diodrast-I^{131}, p-aminohippuric acid (PAH), creatinine, and inulin carboxylic acid-C^{14} were measured in the pithed Necturus from which samples of branchial arterial blood and of cloacal urine were obtained. Close correspondence between simultaneous creatinine and inulin clearances agrees with Bott's evidence (Am. J. Physiol. 168:107, 1952) that either substance may be used to estimate glomerular filtration rate. Diodrast clearance is less than creatinine clearance at low plasma concentrations of diodrast and approaches creatinine clearance at high concentrations. With less than 1 mg diodrast/100 ml plasma, the diodrast/creatinine clearance ratio is independent of concentration. This ratio exhibits seasonal variation, averaging 0.2 in "winter" animals and 0.5 in "summer" animals. These results are consistent with tubular reabsorption of diodrast. Comparison of PAH and diodrast clearances indicates that these substances compete for reabsorption. Furthermore, under conditions of low diodrast and very high PAH concentrations the diodrast/creatinine clearance ratio increases to about 2.0 while the PAH/creatinine ratio remains unity. All data obtained are consistent with the theory that observed renal tubular transport of diodrast in Necturus is the net result of simultaneous transport by 1) a reabsorptive pathway shared with PAH, and 2) a secretory pathway partially shared with PAH. (Supported by Army Contract DA-49-007-MD-624.)

316. FORMATION OF ADRENALINE FROM NORADRENALINE. Norman Kirshner and McC. Goodall (intr. by Keith S. Grimson). Depts. of Biochemistry and of Physiology, Duke Univ. School of Medicine, Durham, N. C.

It has been demonstrated that the methyl group of methionine can be utilized in vivo for the formation of adrenaline (Keller et al. J. Biol. Chem. 183:627, 1950). Similarly, C-14 labeled adrenaline has been isolated from the adrenal gland of the rat after injection of C-14 labeled noradrenaline (Masuoko et al. Proc Soc. Exptl. Biol. & Med. 93:5, 1956). Bullbring (Brit. J. Pharmacol. 4:234, 1949), using a pharmacological assay, reported the formation of adrenaline by homogenates of dog and cat adrenal glands in the presence of ATP. Goodall and Kirshner (J. Biol. Chem., in press) have shown that adrenal slices formed noradrenaline and adrenaline, in sequence, from tyrosine and dopa. It is now shown that the soluble fraction of homogenates of adrenal medullae form adrenaline from noradrenaline and methionine in the presence of ATP. Bovine adrenal medullae were homogenized in ice cold 0.25M sucrose and centrifuged for 20 min at 20,000 x g. The supernatant was decanted and the sediment was washed and resuspended in 0.25M sucrose. The supernatant fraction and the particulate fraction were each incubated in a solution containing phosphate buffer, pH 7.4, 50 μM; reduced glutathione, 1 μM; $MgCl_2$, 200 μM; ATP, 15 μM; L-noradrenaline, 2.5 μM; and L-methionine-methyl-C-14, 1 μM (SA 1 μc/μM); final volume 2 ml. Incubation was for 3 hr at 37° under nitrogen. Adrenaline and noradrenaline were separated by ion exchange chromatography and assayed for radioactivity. Radioactivity was found only in those adrenaline fractions obtained from incubations containing supernatant. The active enzymes can be recovered in the precipitate resulting from half-saturating the supernatant fraction with ammonium sulfate. (Supported by the Natl. Insts. of Health Grant H-2546.)

317. FREE ENERGY, HEAT AND ENTROPY CHANGES OF GLUTAMINE AND ADENOSINE TRIPHOSPHATE HYDROLYSIS. C. Kitzinger, T. Benzinger and R. Hems (intr. by O. Gauer). Naval Med. Research Inst., Bethesda, Md., and Dept. of Biochemistry, Univ. of Oxford, England.

Since Meyerhof's measurement in 1932 it was accepted that the free energy and heat changes of ATP hydrolysis were of similar magnitude, approximately -12,000 cal/mole. A new method of microcalorimetry enabled us in 1954 to remeasure, on materials prepared by Morales and Podolsky, the heat change of ATP and to find $\Delta H = -4{,}800$ cal/mole (Z. Naturforsch. 10-b, 375, 1955). The questions of free energy and entropy remained unsolved. We therefore tried to replace in the estimate of Levintow, Meister and Morales the assumption of analogy between glutamine, asparagine, glutamic and aspartic acids with direct measurements of heats of hydrolysis and ionization of these compounds, and thereby to confirm their estimate. However, we found the heats of transition from the amides to the undissociated acids different by more than 1000 cal/mole. Therefore, a direct approach, calorimetric determination of the glutaminase equilibrium was taken, applying the theory advanced by Benzinger (Proc. Natl. Acad. Sci. 42:199, 1956). With powerful preparations of glutaminase and increased sensitivity of heat measurements (recording the conversion of 10^{-9} M/sec) the reversibility of glutamine hydrolysis could be observed. The equilibrium in 0.9 M NH_4 glutamate was found to be: $k_c = 950$. With activity coefficients calculated by Spencer and Bascombe, $k_{298} = 400$, $\Delta F'^{\circ}_{298} = -3520$ cal/M, $\Delta F'^{\circ}_{310} = -3430$ cal/M. Accepting the value of Levintow and Meister for the glutamine synthetase reaction (at pH 7.0): $\Delta F'^{\circ}_{310} = -4300$ cal/M, the data for ATP hydrolysis are as follows: $\Delta H = -4800$ cal/M, $\Delta F'_{310} = -7730$ cal/M and $\Delta S' = +9.45$ e.u.

318. INITIAL EFFECTS OF COLD EXPOSURE UPON UPTAKE AND RELEASE OF THYROIDAL I^{131} IN HAMSTERS. Karl M. Knigge and Robert S. Goodman (intr. by J. D. Green). Dept. of Anatomy, Univ. of California, Los Angeles.

During the first 12 hr of exposure to 5°C, hamsters exhibit an accelerated release of thyroidal I^{131}, followed by a period of complete inhibition. After 24-36 hr of cold, release is resumed at a rate which may be faster than normal. Twelve-hour I^{131} uptake is decreased approximately 55% of normal during the first 12 hr and not significantly after 24 hr of exposure. Significant increase in uptake is found after 3 days of cold. The degree of cold does not appear to affect the magnitude of the depression of I^{131} uptake,

inasmuch as similar depressions are observed during exposure at 5° or 0°C. Removal of the pituitary gland 24 hr prior to cold exposure abolishes the depression in uptake. Animals at room temperature exhibit normal or slightly increased uptakes 12-24 hr after hypophysectomy. Ligation of the renal blood vessels and ureters likewise abolishes the depression in uptake, while adrenalectomy does not prevent this phenomenon. The depression in I^{131} uptake during the initial stages of cold exposure appears to be due to an increase in renal elimination of iodide. This renal mechanism is affected to the same degree by cold of 5° and 0°C, is independent of the adrenal glands but appears to require the presence of the pituitary.

319. LIPEMIA OF PREGNANCY IN THE RAT. E. Knobil, M. C. Hagney* and N. R. Lampert.* Dept. of Physiology, Harvard Med. School, Boston, Mass.

The finding of a striking lactescence in the sera of pregnant rats near term led to an investigation of serum total lipid, total cholesterol and phospholipid concentrations during pregnancy in the rat. It was found that these constituents decreased slightly between the 1st and 18th day of gestation; rose suddenly on the 19th day reaching peak concentrations immediately prior to parturition on the 22nd day and returned to control values on the 3rd day post partum. Maximal increases ranged from approximately 900% for total lipids to 150% for cholesterol and phospholipids. At term, corresponding fetal serum lipids were 25% of the maternal values, suggesting partial placental impermeability to circulating lipids. These alterations in serum lipid concentrations could not be accounted for by increases in food intake during the latter part of gestation as shown by pair-feeding experiments. Increases in serum lipid concentrations were not observed in pseudopregnant rats. Ovariectomy on the 19th day of pregnancy did not abolish the lipemia if live fetuses were present in utero at autopsy on the 21st day of gestation. Removal of fetuses on the 15th and 19th days leaving the placentae in situ reduced serum lipid concentrations on the 22nd day when compared to pair-fed laporotomized control pregnant rats. The remaining placentae, however, were reduced in size and appeared abnormal in other respects. Injections of placental homogenates and saline extracts of placenta into non-pregnant rats failed to induce a rise in blood lipids.

320. CHOLINESTERASE ACTIVITY OF FROG SKIN. Daniel C. Koblick (intr. by B. T. Scheer). Dept. of Biology, Univ. of Oregon, Eugene.

Recent observations on systems which actively transport sodium ions have implicated cholinesterase in the transport process (Kirschner. Nature 172:348, 1953; Van der Kloot. ibid., 178:366, 1956). The present work was undertaken to demonstrate the presence of the enzyme in frog skin, a material widely used for the study of active sodium transport. Using acetylcholine chloride (2×10^{-2} M) as substrate, unpigmented ventral skin from R. pipiens was found to possess a cholinesterase activity of 3 - 5 μl CO_2/hr/mg N. The tela subcutanea, obtained by dissection, possessed an activity of 60-75 μl CO_2/hr/mg N. Estimates based on the N content per unit area of whole skin and of tela subcutanea indicate that more than 90% of the activity is located in the latter. Measurement of activity at substrate concentrations in the range 4×10^{-3} M - 0.125 M reveal that the enzyme is of the serum type ("pseudocholinesterase" of Mendel). No substrate inhibition occurs, even at the highest substrate concentrations studied. Butyryl choline and benzoyl choline are readily hydrolyzed, but acetyl β-methyl choline is not. (Supported by a grant from the Natl. Science Fndn.)

321. NATURE OF LONG LASTING EFFECTS PRODUCED BY A SINGLE PULSE STIMULATION OF THE RETICULAR FORMATION. K. Koizumi,* I. Suda* and C. McC. Brooks. Dept. of Physiology, State Univ. of New York Med. Ctr., New York City.

Single 5-8 msec rectangular pulses applied to the suprabulbar reticular formation produced a brief (lasting 20-30 msec) inhibition followed by facilitation (lasting 90-100 msec) of the monosynaptic reflex in the cat. Removal of the cerebral cortex and even decerebration produced no change in some animals but in others the facilitatory actions of the stimulus were reduced with an accompanying prolongation of the initial inhibition. Ablation of all parts of the cerebellum except the lateral portions (ansiform, parafloccular lobes) also reduced facilitation and prolonged the inhibitory effect. Removal of the remainder of the cerebellum, however, increased and prolonged the facilitation resulting from reticular formation stimulation and reduced and shortened inhibitory actions. Section of the 8th nerve minimized both effects of reticular formation excitation. Ipsilateral and contralateral section of the spinal roots had no effect on these reticular formation actions in some cases and reduced facilitation in other instances. Curarization likewise did not affect the higher center's influence on the monosynaptic reflex. Interaction with the higher centers mentioned and reverberation from the periphery have been eliminated as possible factors in the long-lasting effects of reticular formation stimulation. Recordings from single fibers in the lateral columns revealed descending discharges during and following reticular formation stimulation but they persisted for only 20-30 msec. (Supported by grant PHS-B-847c from the Natl. Inst. of Neurological Diseases and Blindness, PHS.)

322. CORTICAL SPREAD OF AFTER-DISCHARGE TO INDUCED SEIZURE IN MONKEY. B. Konigsmark and J. D. French (intr. by R. Sonnenschein). VA Hosp., Long Beach, and Univ. of California, Los Angeles.

Seizures were induced in 12 curarized monkeys by applying a stimulus (50 cps, 0.5 msec, 1-15 v) to motor leg and arm areas of exposed cortex. Bipolar recordings were made from multiple cortical loci to note extent of diffusion of induced seizures. These experiments demonstrated that: 1) threshold and diffusion of seizure induced at a given point is reproducible. 2) Duration and dissemination of induced seizure correlated, generally, with stimulus intensity. 3) In general, induced seizure spreads locally to contiguous cortical zones. Such discharge appeared as the well known sustained or intermittent spiking. 4) Areas marginal to zones exhibiting after-discharge to cortical stimulation display slow wave activity; these areas become involved in seizures induced by higher stimulus intensities. 5) Contralateral cortical spread of induced seizure begins at point homotopic to induced discharge and spreads with pattern similar to ipsilateral spread, but with somewhat less vigor. 6) Cutting corpus callosum prevents spread of induced seizure to contralateral cortex, but does not change pattern of ipsilateral spread. 7) Metrazol does not change pattern of induced seizure spread, but lowers threshold and markedly prolongs duration and extent of seizure. The implications of these findings will be discussed.

323. ROLE OF DOG'S SPLEEN IN OVERTRANSFUSION. Leon Kraintz,* E. L. Smith, R. A. Huggins

and M. Fullen.* Univ. of Texas, Dental Branch, and Baylor Univ. College of Medicine, Houston.

We have published data on dogs bled to produce hemorrhagic shock and found no evidence of release of erythrocytes from reservoirs into the circulation. This is contrary to the concept that the spleen serves as an important blood reservoir. These observations concerned the total erythrocytes released from reservoirs but not specifically by the spleen. The present experiments were designed to determine whether erythrocytes were "trapped" in the spleen during overtransfusions, since we have previously demonstrated that there was a disappearance of red cells after overtransfusions. Anesthetized control dogs were given radiochromate labeled erythrocytes. After 20 min repeated samples of arterial and venous blood were taken and the radioactivity determined as counts/ml of packed cells. One hour later the spleen was surgically exposed and the entire splenic circulation clamped immediately. Samples were obtained from the splenic vein by syringe aspiration to apparent exsanguination of the organ. Another group of anesthetized dogs was overtransfused with blood containing radiochromate labeled red cells. The same sampling procedures were used as with the controls. If the spleen functions as a reservoir, one would find differences between the counts/ml of the packed erythrocytes of the splenic blood and that of samples from the general circulation. Overtransfusion should accentuate this difference. No differences were found in the arterial, venous and splenic blood in either the control dogs or the overtransfused group. The results obtained in the experiments do not support the contention that the spleen has a reservoir function.

324. CIGARETTE SMOKING AT ALTITUDE. C. H. Kratochvil, S. S. Wilks and W. A. Gerrard, III (intr. by Robert T. Clark). Dept. of Physiology-Biophysics, School of Aviation Medicine, USAF, Randolph AFB, Texas.

The discovery of substantial quantities of carbon monoxide in some post-mortem tissues raised the question of the effect of cigarette smoking at altitude. It was felt essential to determine 1) whether a significantly different amount of carboxyhemoglobin was formed when smoking at an altitude of 18,000 ft and 2) whether performance at this altitude was impaired while smoking. Accordingly, 20 subjects were examined in each of 2 groups. In the first group, blood CO was measured at ground level before and after smoking one cigarette and again at 18,000 ft (altitude chamber) before and after smoking one cigarette. In the second group, performance on the SAM complex coordinator was measured at ground level, at 18,000 ft and at 18,000 ft while smoking. The results indicated that there was no increase in blood CO at altitude as compared with ground level controls after one cigarette. Similarly, there was only a slight decrement in performance when smoking while hypoxic at altitude was compared with the hypoxic controls. The primary physiological hazard in smoking at altitude appears to be hypoxia. There is little exaggeration of the effect from cigarette smoking beyond that caused by the usual amount of carboxyhemoglobin formed.

325. SUPPLEMENTAL FEEDING AND THERMAL COMFORT DURING SLEEP IN THE COLD. M. B. Kreider and E. R. Buskirk (intr. by Austin Henschel). Environmental Protection Research Div., Quartermaster Research and Devel. Ctr., Natick, Mass.

Men seldom maintain body temperature during sleep in extreme cold and frequently awaken within 3-5 hr. Specific Dynamic Action (SDA) of food is known to elevate metabolism and thus body temperature. Ingestion of food prior to retiring would therefore be expected to enhance maintenance of body temperature and rest when sleeping in the cold. Six men were studied while sleeping in Arctic Sleeping Bags at -34.5°C (-30°F). The men ate the usual 3 meals/day at conventional times plus a supplement of 0, 600 or 1200 cal 10 min before retiring. The composition of the supplement was 40% fat, 20% protein and 40% carbohydrate. The following measurements were made during the night: rectal temperature (T_r), skin temperature (T_s) and oxygen consumption ($\dot{V}_{O_2}$). When a 600- or 1200-cal supplement was given T_r, T_s and $\dot{V}_{O_2}$ were significantly higher than with no supplement. There appeared to be no difference between the effect of a 600 or 1200 cal supplement. The supplement also reduced the number of periods of wakefulness and the subjective discomfort associated with cold.

326. COMPARATIVE MEASUREMENTS WITH A NEW PROCEDURE FOR MEASURING THE BLOOD OXYGEN TENSION IN VITRO, USING CLARK'S PLATINUM ELECTRODE. F. Kreuzer and T. R. Watson, Jr. (intr. by S. M. Tenney). Dept. of Physiology, Dartmouth Med. School, Hanover, N. H.

A new polarographic procedure for measuring the blood oxygen tension in vitro has been developed. (Kreuzer. Experientia, in preparation). The equipment consists of a constant temperature electrode; (oxygen electrode of Clark) chamber, polarographic circuit and galvanometer. The chamber contains the lower end of the electrode, a thermometer, and a vertical gas outflow tube. The heparinized blood sample (5-7 cc for each determination) is drawn anaerobically into a syringe and introduced into the chamber at 37°C; the filled chamber is closed at the gas outflow tube. The galvanometer reading is taken at 0.6 v; during the reading the electrode is shaken gently by hand which results, after a few seconds, in a constant and well-defined value. After opening the gas outflow tube, the calibration curve is taken on the same sample, by bubbling through air, nitrogen and oxygen, respectively, until equilibrium is reached; the calibration readings are taken again with closed gas outflow tube and shaken electrode. The calibration curve is linear. In general 3 sample readings and 3 calibration curves are determined which agree closely. Calculated P_{O_2} values thus obtained were compared with 1) adjusted tonometer equilibrations with determination of O_2 in the gas phase by the Van Slyke technique, and 2) data obtained from determinations by the Riley technique. These comparisons yielded agreements with the tonometer values within range of ±5 mm Hg and with the Riley values within ±3 mm Hg.

327. EFFECT OF GRAM NEGATIVE ENDOTOXIN ON THE PULMONARY CIRCULATION. Hiroshi Kuida and Lerner B. Hinshaw (intr. by J. T. King). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

The pulmonary circulation is not considered to play an important role in bacteremic shock. However, experimental studies of local, regional and general hemodynamic responses to endotoxin have not included effects on the pulmonary circulation. The present study was prompted by the incidental but frequent occurrence of ventilatory and cardiac complications in animals and heart-lung preparations following the administration of endotoxin. Fifteen experiments were performed in dogs and cats including intact

preparations and open-chest preparations with and without controlled cardiac output, and 4 studies were performed on isolated, nonventilated dog lungs perfused with a pump-oxygenator system and continuously weighed. E. coli endotoxin was used throughout. Pulmonary artery, pulmonary artery wedge and left atrial pressures were measured in all preparations. Small and large pulmonary vein pressures were also recorded in preparations where these vessels could be catheterized. Results showed qualitative uniformity although there was considerable quantitative variation from preparation to preparation. Left atrial and large pulmonary vein pressures remained constant, whereas pulmonary artery, wedge and small vein pressures and lung weight rose following a variable delay period. The most significant effect of endotoxin was the increase in small vein resistance. Pulmonary arteriolar resistance seemed to remain constant or increased only slightly. Changes in weight in the isolated perfused lung proved to be a sensitive index of arteriolar and venular constriction produced by epinephrine and histamine respectively, the former being accompanied by a fall and the latter by an increase in weight.

328. BONE MARROW FUNCTION IN ISOLATED PERFUSED HIND LIMBS OF RATS. Samuel Kuna,* Albert S. Gordon and Harry A. Charipper. Dept. of Biology, Graduate School of Arts and Science, New York Univ., New York City, and Merck Inst. for Therapeutic Research, Rahway, N. J.

A technique has been developed for the assessment of bone marrow function in isolated perfused hind limbs of rats. A Dale-Schuster perfusion pump was used to recirculate a minimum volume of 15 ml of perfusate through the system. When whole homologous blood was employed, no rigor or edema developed in the standard 4-hr perfusion period. Differential cell counts and determinations of total cellularity were made of the femoral marrows of both the perfused limbs and the unperfused contralateral limbs. Evidence for the maintenance of normalcy in the perfused limbs was apparent from various tests. The femoral marrows within isolated limbs of rats showed significant increases in the concentrations of nucleated R.B.C. when perfused with whole blood obtained from rats subjected to 1) low barometric pressures, 2) hemorrhage or 3) phenylhydrazine treatment. Marrows in limbs subjected to hypoxic stimuli showed even greater erythropoietic stimulation when perfused with whole blood. Studies are in progress dealing with the effects of extra glucose, phosphorylated compounds and antimetabolities upon hematopoiesis in the isolated perfused limb. (Supported in part by grant GS-434 from the Natl. Science Fndn.)

329. RESPIRATORY EFFECTS OF .08 AND .8 ATMOSPHERES OF INSPIRED pO_2 AT A "CONSTANT" ALVEOLAR pCO_2 OF 43 mm Hg. C. J. Lambertsen, H. Wendel,* H. Chiodi* and S. G. Owen.* Lab. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia.

Eight, 21 and 80% O_2 were administered at 1.0 atm. to 6 normal subjects maintained at an alveolar pCO_2 of 43 mm Hg. In the absence of alterations in arterial pCO_2 or pH, 8% O_2 produced a 26, and 80% O_2, a 15% increase in respiratory min volume above the control values obtained during 21% O_2 breathing. The respiratory stimulation associated with 80% O_2 breathing was accompanied by a significant 1.6 mm Hg rise in cerebral venous pCO_2, reflecting a central hypercapnea which may have produced the "oxygen" hyperpnea. Since neither cerebral circulation nor cerebral metabolism was altered by 80% O_2, the internal jugular venous hypercapnea was due to diminished hemoglobin reduction. The hypoxic hyperventilation of 8% O_2 breathing was associated with a 5 mm Hg fall in cerebral venous pCO_2, due to increased rate of brain blood flow. It is therefore possible that, in spite of a fixed arterial pCO_2, the chemoreflex respiratory response to hypoxia is in part counteracted by a fall in central stimulus level, brought about by hypoxic cerebral vasodilitation.

330. EFFECT OF UNILATERAL ANOXIA ON PULMONARY CIRCULATION. F. J. B. Lanari-Zubiaur (intr. by W. F. Hamilton). Depts. of Physiology and Pharmacology, Med. College of Georgia, Augusta.

Blood flow in the two lower lobes was measured under unilateral anoxia with spontaneous breathing and natural blood supply at equal pressure. The chest was opened and a tube was tied into the left stem bronchus. The other lung was separately ventilated through the trachea. The veins from the lower lobes of the 2 lungs were cannulated and the drainage led into the intrathoracic right jugular vein by means of a wide plastic tube. The chest was closed and spontaneous respiration established in the prone position. As soon as the respiratory rate had become constant and the color of the blood in the tubing normal, flow was measured by diverting the drainage simultaneously by means of T tubes and pinch clamps into 2 graduates for 10 sec and returning the blood through the left jugular. Measurements were made with both lungs receiving room air as a control. The 2 separate airways were then connected to 2 Sanborn spirometers, one containing O_2 and the other N_2, with CO_2 absorption. The flow measurements were repeated. After a few measurements the animal was returned to air and the airways reversed. Blood coming from the nitrogen lung became black in 30 sec. The flow from the anoxic lung showed no consistent change while the flow through the oxygenated lung was consistently increased. (Program sponsored by the Natl. Heart Inst. and the American Heart Assoc.)

331. BLOOD SUGAR LEVEL AND VISUAL FLICKER-FUSION THRESHOLD FOLLOWING INSULIN COMA. Carney Landis, D. Dillon,* J. Leopold* and J. Rutschmann.* Psychology Dept., New York State Psychiatric Inst., New York City.

It is known that the critical threshold of flicker-fusion (CFF) is reduced during hypoxia, hypercapnia and increased blood alcohol. We have previously demonstrated reduced CFF in approximately 75% of patients following insulin coma and/or electric consulsive therapy. Others have shown a strong tendency for reduced blood sugar during and shortly after coma although some patients gave no reduction. From 30 patients receiving insulin coma therapy we obtained measures of CFF, reaction time, tapping speed and blood sugar level (Folin-Wu method) at intervals during the course of treatment. This report concerns results obtained when the tests were applied within 1 hr after coma. The number of comas which the patients had had before the tests varied from 10-50. The reaction time and tapping speed gave no constant direction of change. Of 91 blood sugar determinations, 69% were lower in mg/% than they had been at 6:00 a.m. before coma. Of 91 CFF determinations, 79% were more than 1 cps lower after coma than they had been at final determination before coma series was started. In 57% of instances (or in 12 of 30 patients), the direction of change of these two measures was the same. We conclude that neither blood sugar level nor CFF constitutes a uniform criterion of the fact of a recovery from insulin coma although CFF is

a somewhat better indicator. (This project has been conducted under Grant M-872, Natl. Insts. of Health, PHS.)

332. HARMONIC ANALYSIS OF PRESSURE WAVE PROPAGATION. Milton Landowne. Gerontology Branch, Natl. Heart Inst., Bethesda, and Baltimore City Hosp., Baltimore, Md.

Modification in the form of the central arterial pressure pulse, as it travels peripherally, indicates that there are differences in propagation velocity, damping and/or reflection of some components. We have found (Fed. Proc. 12:82; 13:83) that wave velocity and damping in the human brachioradial artery are both pressure and frequency dependent. Porjé's harmonic analysis of syphygmograms concludes that significant frequency dependent wave reflection occurs in the aorta. We have some reservations in assuming that the actual components generating the arterial pressure pulse are represented by harmonic analysis of a single beat. Nevertheless, we have used the method—after finding results to be reproducible and to be consistent with observations on induced sinusoidal waves of comparable frequencies. Using a schema for 48-ordinate harmonic analysis, in pressure records obtained from the aorta and subclavian artery (23 pulses in 3 subjects), amplitudes of harmonics above the 3rd were less than 5 mm Hg and less than 2 mm Hg above the 5th. Over aortic distances of 10-60 cm (9 segments), the phase velocities varied with frequency and location, and were consistently higher for the lowest frequency components. In the brachioradial artery (8 pulses, 4 subjects), phase velocity was also sometimes higher for the lowest frequency components than for higher harmonics. Compared with the aorta, but in different subjects, the brachioradial artery showed slightly greater harmonic content at higher frequencies. These observations confirm Porjé, and are appropriately explained by the hypothesis that reflected components may contribute significantly to phase and amplitude.

333. DOSE-RESPONSE RELATIONSHIP TO HISTAMINE IN THE CHRONIC GASTRIC FISTULA RAT. Ardelle Lane (intr. by S. C. Harris). Depts. of Physiology, Dental School, Northwestern Univ., and of Clin. Science, Univ. of Illinois, Chicago.

The chronic gastric fistula rat responds to histamine (Am. J. Physiol. 187:1956). This study was undertaken to ascertain if a dose-response relation exists. The animals were fasted for 14 hr in a restraining cage; then the stomach was washed and hourly collections started. An indewelling needle was inserted s.c. and every hour 3 cc of normal saline was injected to compensate for the water and chloride loss. After 4-6 hourly control collections, the dose of histamine diphosphate was injected in 3 cc of normal saline and the hourly collection of gastric juice and injection of 3 cc of normal saline were continued for 5-6 hr. The mg HCl output for the hour before and after the injection of histamine was compared, the results being as follows: the percentage increase in output to 0.25 mg (histamine diphosphate) was 67%; to 0.5 mg, 91%; to 1.0 mg, 200%; to 2.0 mg, 233%; to 3.0 mg, 218%; and to 4 mg, 137%.

334. RAPID CONTINUOUS MEASUREMENT OF LEG BLOOD FLOW IN MAN: EFFECTS OF VASODILATOR DRUGS. Mark H. Lane,* Darab K. Dastur,* Douglas B. Hansen* and Louis Sokoloff. Natl. Inst. of Mental Health, Bethesda, Md.

A method applying the Fick principle to the rapid continuous measurement of total leg blood flow in man and utilizing Na^{24} as the tracer material has been previously described (XXth Int. Physiological Congress, Brussels, 1956). This method has been employed in studies of the continuous effects of intra-arterial injections of two vasodilator drugs, tolazoline hydrochloride (Priscoline) and nylidrin hydrochloride (Arlidin) on the leg blood flow of normal man. Following a control period of between 5 and 10 min either 50 mg of tolazoline hydrochloride or 10 mg of arlidin hydrochloride were injected slowly over a period of 1-2 min into the femoral artery of the experimental leg. Continuous measurement of total leg blood flow was made throughout the control period and throughout the entire duration of drug action. Values for blood flow obtained were for the entire leg but were corrected to ml blood/100 ml leg/min following measurement of total leg volume by means of a water displacement technique. Both drugs, when administered in this manner, were found to increase markedly and abruptly the leg blood flow, the increases ranging from 2-10 times that of the control value. In all cases, however, the duration of action of the drugs was limited returning to control levels within 20 min following completion of the drug injection. The Na^{24} method was found suitable for following continuously the rapid changes in leg blood flow produced by the administration of these drugs.

335. COUPLED ELECTRICAL BEATS AND OTHER BIOELECTRIC PHENOMENA IN PREGNANCY AND LABOR. S. D. Larks (intr. by W. A. Selle). Dept. of Biophysics, School of Medicine, Univ. of California, Los Angeles.

With further development of the electrohysterographic studies previously reported, the phenomenon of coupled electrical beats has been observed. The contractio bigemina uteri has certain points of resemblance with the bigeminal pulse originally described by Traube for the heart. The second member of the coupled pair has a waveform similar to the ventricular extra-systole, and long pauses separate the pairs. The significance of such pairing in terms of the effectiveness of uterine contractility is not yet clear. If, as seems probable, it should turn out that there exist for the uterus two basic types of contractile activity, e.g. first the controlled beat originating in a pacemaking area, and second the ectopic beat originating in the muscle mass, further light could be thrown on the fundal dominance concept of Reynolds and the Triple Descendent Gradient of Caldeyro-Barcia. Long steps could be taken toward the comprehension of those difficulties in labor which go by the names of incoordination and uterine inertia. Extension of the electrical studies into the gestational period indicates that recordable electrical activity may be seen early in pregnancy. Infrequent yet recognizable contraction patterns have been noted in middle pregnancy, while in the late 3rd trimester, around the 36th and 37th wk of pregnancy, a high level of electrical activity is seen. At this time some complexes are seen which resemble those of labor, thus heralding the approach and onset of labor. It is not known whether there is a uniform increase in recordable electrical activity through pregnancy, or whether cycles of activity exist which can be correlated with changes in hormone levels. (Supported by research grant PHS, RG 4462.)

336. EFFECT OF VARIOUS TYPES OF FLUID THERAPY ON SURVIVAL OF MONKEYS EXPOSED TO A STANDARDIZED SCALD. Sigmund E. Lasker* and Charles L. Fox, Jr. Depts. of

Surgery and Physiology, New York Med. College, Flower and Fifth Ave. Hosps., New York City.

A standardized edema-forming fatal scald injury with gross appearance and subsequent chronological changes, similar to the full-thickness burns frequently seen in man, was produced in the macaca mulatta and macaca irus. Non-fatal burns were observed for ± 30 days. The scald involving 55% of body surface is produced by dipping manually the shaven ether-anesthetized animal supported in an erect position, into water at 75°C for 15 sec to the level of the xiphoid process. Eleven untreated monkeys weighing an average of 4.3 kg survived for an average of 9.6 hr. Forty-four animals weighing an average of 3.7 kg were treated with up to 150 ml/kg body weight of a 0.14 molar balanced electrolyte solution alone or combined with blood or dextran given intravenously, and the 0.14 molar and 0.07 molar solutions given orally. Fluids were given in 2 doses over a period of 4 hr for each dose beginning 1-2 hr postburn and 24 hr later. Survival increased to an average of 3.0 days for all groups with no important differences among the groups. With less severe burns permitting survival for 3-8 days, early fluid therapy did not appear to prolong life. (Supported by the Research and Development Div., Surgeon General's Office, Dept. of the Army.)

337. EFFECT OF SECOND-STAGE LOBECTOMY ON PULMONARY FUNCTION OF DOGS. M. T. Lategola, W. H. Massion and J. A. Schilling (intr. by A. N. Taylor). Depts. of Physiology and Surgery, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y., and Univ. of Oklahoma School of Medicine, Oklahoma City.

Forty days following the removal of the 3 lower lobes of the right lung, the 2 upper lobes of the left lung were removed in 5 dogs. The latter procedure constitutes a removal of 15.4% of the original whole lung and both procedures additively total 56.5% of the original whole lung. Measurements were made post-operatively on the 10th, 40th and 80th days. The total O_2 uptake was insignificantly changed. Relative to the original % lobar weights (as reported by Rahn et al., J. Appl. Physiol. 8:417, 1956) the remaining right upper lobe showed an increase in O_2 uptake and tidal volume whereas the remaining left lower lobe manifested a decrease in both these parameters. Mean pulmonary arterial pressure was markedly elevated. Both the total functional residual volume and static total-chest compliance (at 20 cm H_2O pressure) were reduced. An analysis based on the amount of gain in function per unit gain in weight of the two remaining lobes revealed that the right upper lobe is decompensating at this stage whereas the left lower lobe may not as yet have reached a maximum in compensation. (Supported by funds from the Aviation School of Medicine, Randolph Field, Texas; AF Contract #41-(657)-105).

338. CALIBRATION OF AN AORTIC CIRCUMFERENCE GAUGE. R. W. Lawton and C. C. Collins.* Aviation Med. Acceleration Lab., Naval Air Development Ctr., Johnsville, Pa., and Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia.

The pressure circumference relation for the aortic arch in situ has been described (Rushmer et al. Am. J. Physiol. 183:545, 1955). In this study, gauges made of fine rubber tubing (0.5 mm i.d.) filled with mercury and sealed at both ends with electrodes and insulated leads were stretched around the aorta. Aortic circumference was registered as a change in electrical resistance of the mercury column. The gauges (3.5 cm long, resistance of 0.8 ohms), had a sensitivity of approximately 1.5 mv/mm elongation as one leg of a Wheatstone bridge with a 6-volt supply. Static calibration was linear to 15% extension. Slow creep behavior occurred under sustained stress. Free longitudinal resonant frequency was 5-17 cps depending upon load. Frequency-amplitude plots of resistance vs. longitudinal sinusoidal gauge displacement show a gradually rising amplitude above 30 cps, perhaps due to a radial mode of vibration of the tube. No clear resonance was seen. Above 10 cps resistance shows a phase lead over displacement. Amplitude nonlinearities become significant at gauge displacements of 5% with initial bias stretches up to 15%. Compensation by a phase shifting network produces a flat response to 150-200 cps. Data on the dog's aorta using the compensated and uncompensated gauge are compared.

339. EFFECT OF HEPATIC ARTERIALIZATION UPON BILIARY SECRETION. S. H. Lee, Morton Levine, E. J. Fedor and Bernard Fisher (intr. by T. K. Kruse). Dept. of Surgery, Univ. of Pittsburgh, Pittsburgh, Pa.

It had previously been reported by us (Fisher et al. Surgery 38:181, 1953) that 10% of animals with arterialized livers died of acute cholecystitis and/or common duct obstruction. Since no information was available concerning the effect of permanently increasing the hepatic blood flow on biliary secretion this was undertaken using a modified Thomas-type fistula to collect daily samples of bile (A. M. & P. M.). a) Bile volume: both normal and arterialized animals show a greater bile volume in the morning than in the afternoon, but permanently increasing the blood supply 2- to 3-fold does not alter the volume of bile secreted. For example, 63 hourly collections of bile in 13 normal animals between 9 and 10 a.m., averaged 11.2 ml or 1.0 ml/kg in volume while 30 similarly collected samples from 7 arterialized dogs had a mean volume of 11.5 ml or 1.0 ml/kg. b) Cholic acid: although the total amount of cholic acid in bile varied from animal to animal and from day to day in the same animals, when expressed in terms of mg/cc/kg, a uniform value was obtained; 2.8 in the a.m. and 2.2 in the p.m., for normal animals. Arterialized animals averaged a slightly lower output—2.0 and 1.5 mg/cc/kg in the a.m. and p.m. respectively. c) Cholesterol: in normal animals there was 0.16 and 0.14 mg/cc of cholesterol in the a.m. and p.m. samples of bile while in the arterialized group this was 0.10 and 0.09 mg/cc. d) Bile pigments: total bilirubin in 63 hourly collections in 13 normal dogs was 1.6 and 3.2 mg/cc in the normals and 2.28 and 2.86 mg/cc in those arterialized. It is concluded that little difference in bile volume or composition results from permanently increasing the blood flow 2-3 times through the liver. (Supported in part by Grant A-369(C3) from the Natl. Insts. of Health, PHS.)

340. BODY SODIUM AND POTASSIUM IN RATS RECEIVING INCREASED DIETARY SODIUM CHLORIDE. Janet Lemley-Stone,* William J. Darby and George R. Meneely. Radioisotope Service and Research Lab., Thayer VA Hosp., and Depts. of Biochemistry and Medicine, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Hypertension, renal damage and shortened life span occur in rats fed high levels of NaCl. Increased dietary K affords some protection against NaCl toxicity as evidenced by longer survival and decreased incidence of hypertension. Exchangeable body Na was measured in rats after 8 months on diets

containing 10% NaCl with either 0.7% or 5.6% KCl. Controls were fed diets containing either 0.2 or 1.1% NaCl and 0.7% KCl. Rats on high Na-low K had more body Na than either controls or those fed high Na-high K. After 12 months on the diets, rats fed high Na-high K had more exchangeable body K than animals eating high Na-low K. The rats on high Na-low K had 9 mEq more Na/kg of lean body weight and 13 mEq less K than those fed high Na-high K. Both groups on high Na intakes with either high or low dietary K had more body K than rats fed normal levels of Na. Myocardial and hepatic tissue from rats fed elevated Na with either high or low K contained more K than tissue from controls. Muscle from high Na-low K fed animals contained more Na and less K than from high Na-high K fed rats, while muscle from controls was intermediate between these 2 experimental groups. (Supported in part by research grant H-1816 (C2), Natl. Heart Inst., PHS.)

341. SEPARATION OF A SYSTEM FOUND IN PLASMAS OF PATIENTS WITH ESSENTIAL HYPERTENSION. Edward Leonard and Stephen Hajdu (intr. by Ronald Scantlebury). Natl. Heart Inst., Bethesda, Md.

It has been reported that the system under study contains more than one component. Component L in serum is destroyed by heating at 60°, but survives lyophilization. Activity is destroyed if serum pH is lowered to 4.9 at 4° for 2 hr and then returned to physiological pH. It is destroyed at pH 9.5. It is found in the precipitate formed by addition of $(NH_4)_2SO_4$ to 50% saturation at pH 7.5, and precipitates when the redissolved material is dialyzed free of salt. Component S activity in serum disappears after dialysis against water in the cold, frequently in 2 hr, but only if the ratio of serum to outside solution is at least 1:100. At 1:50, activity persists even after prolonged dialysis. The fact that activity disappearance depends on volume ratio in a short dialysis suggests that, unless dialysis conditions cause destruction, component S achieves diffusion equilibrium across cellophane rapidly. The large volume ratio needed indicates that either it is mostly bound to a non-dialyzable component or is free in great excess. The latter possibility is ruled out since no component S occurs in serum ultrafiltrate. Movement of component S across cellophane is shown by dialysis of 1 volume of normal serum against 1 volume of serum without component S, since both sera contain partial activity after equilibration. Serum must therefore contain a non-dialyzable factor (component F) to which component S is bound. On the basis of pH sensitivity, component F is different from component L. All 3 components are probably needed for activity.

342. EFFECT OF POTASSIUM AND CALCIUM DEFICIENCY ON MONOPHASIC ACTION POTENTIAL OF ISOLATED, PERFUSED RABBIT HEART. E. Lepeschkin, B. Surawicz,* H. Herrlich* and B. F. Hoffman. Div. of Exptl. Medicine, Univ. of Vermont, Burlington, and Dept. of Physiology, State Univ. of New York, Brooklyn.

When normal Krebs-Henseleit solution is replaced by the same solution containing no potassium, the descending branch of the ventricular monophasic action potential becomes progressively longer and less steep, leading to complete disappearance of a base line between two successive beats, while the plateau shows little change. At this time the ECG shows a very long, rounded T wave, and ectopic beats usually appear. Later the initial portion of the plateau becomes very steep, corresponding to a decrease in the duration of the upper half of the action potential and to the appearance of a new, short T wave or kink in the S-T segment preceding the wide original T wave in the ECG. Perfusion with calcium-free solution does not influence the steepness of the descending branch of the action potential but increases the duration of the plateau. At this time the ECG shows a long S-T segment without change in the duration of the T wave. After several minutes the plateau and the S-T segment become again shorter. Perfusion with a solution containing no potassium or calcium causes increased duration of both the plateau and the descending branch of the action potential. These observations were made largely on the monophasic action potential obtained by means of the suction electrode, but they were confirmed on the transmembrane potentials obtained with a flexibly mounted intracellular micro-electrode.

343. ELECTROGRAPHIC RECORDINGS OF AMYGDALOID ACTIVITY DURING A CONDITIONED RESPONSE. Henry Lesse (intr. by H. E. King). Dept. of Psychiatry and Neurology, Tulane Med. School, New Orleans, La.

Chronically prepared cats were used to determine the effects of certain induced changes in behavior on electrographic activity from various subcortical regions. EEG recordings were obtained from multiple electrodes stereotaxically implanted. Concurrently, the animals, in a constant environment cage, were exposed to a conditioning technique and to a variety of stimulus situations. Peripheral stimulation of any modality which resulted in excited behavior (manifested by agitated movements, or by organized escape, attack, or defensive reactions) induced a distinct, reproducible and localized change in electrographic pattern. This response (high amplitude, rhythmic, 40-45/sec activity) was recorded regularly from the lateral amygdaloid nuclei and occasionally from the subjacent pyriform cortex, but not from other subcortical areas examined. Stimuli which resulted in waking or alerting responses only did not induce this change in pattern but did elicit typical "desynchronization" in the neocortex. A delayed conditioned response was employed to determine whether an auditory stimulus, ordinarily ineffective, could evoke this electrographic pattern after being repeatedly paired with a nociceptive stimulus. A grid shock to the paws was paired with a buzzer; the shock delivered 6 sec after onset of the auditory stimulus. After repeated pairings the characteristic amygdaloid pattern was induced by the auditory stimulus alone and its development and disappearance was associated with the acquisition and extinction of a conditioned behavioral response. This type of electrophysiological activity, unlike neocortical "desynchronization," may be associated with behavioral responses encompassing "excitement" or "excited attentiveness" as distinct from mere arousal from sleep or alerting to attentiveness. (Supported by N.I.M.H. Career Investigator Grant.)

344. ROLE OF TYROSINE RESIDUE IN INTERACTION OF TESTOSTERONE WITH SERUM ALBUMIN. B. H. Levedahl and Evan Oyakawa (intr. by T. L. Jahn). Dept. of Zoology, Univ. of California, Los Angeles.

Previous studies of albumin-testosterone interaction have shown that if testosterone is bound through a specific chromophoric grouping of the protein, that grouping is most probably the tyrosine residue. To examine this postulation in more detail, serum albumin has been modified by both iodination and by

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ketenization to block the tyrosine residues; and testosterone interaction with these modified proteins has been studied. Iodination to the levels of 10, 32 or 43 atoms of iodine/molecule of protein does not alter significantly the interaction characteristics of testosterone with the protein. More intensive iodination of the protein (135 atoms of iodine/molecule of protein) does reduce the free energy of interaction with testosterone. Ketenization of serum albumin reduces the ability of the protein to interact with testosterone. Using these data, a configurational model has been deduced to describe the mechanism of interaction of testosterone with bovine serum albumin.

345. INTRACELLULAR WATER OF LARVAL TISSUES OF SOUTHERN ARMYWORM. L. Levenbook and Jeanne Kuhn.* Jefferson Med. College, Philadelphia, Pa.

A critical study of the quantitative distribution of solutes between the haemolymph and tissues of insects requires a knowledge of the tissue intracellular water content. The intracellular water content of the isolated gut, fat-body and combined internal organs of the 5th instar caterpillar of the Southern Armyworm have been determined using C^{14}-labeled inulin (Cf. Cotlove, Fed. Proc. 14:32, 1955). The difference between the total water content (from the dry weight) and the determined extracellular water was assumed to be intracellular water. C^{14}-inulin injected into the haemolymph was neither metabolized nor excreted during 24 hr. The haemolymph contained no inulase, nor was the inulin bound to haemolymph proteins. Hence there is good reason to believe that the tissue inulin space is indeed a measure of the extracellular water. The tissues were dissected out in an apolar solvent, Freon 113, superficially dried on filter paper, and divided into 2 weighed portions; the dry weight was measured on the one, and the C^{14} content determined on the other. The intracellular water content was substantially the same whether the analyses were made 2-3/4 or 22 hr after the inulin injection. Expressed as percentage of the tissue wet weight the results were as follows: fat-body, 44.6± 1.8 (S.D.); gut, 54.5± 3.3; combined internal organs, 45.5. (Supported [in part] by a research grant (H-1917) from the Natl. Heart Inst., PHS.)

346. VENOUS CONSTRICTION IN MAN DURING EXPOSURE TO POSITIVE G FORCE. Sidney D. Leverett, Jr., Stuart Bondurant and Mitchell B. Riley (intr. by Edwin P. Hiatt). Aero Med. Lab., Wright Air Development Ctr., Wright-Patterson AFB, Ohio.

To study reflex changes in human venous tone during exposure to positive (head to foot) g forces, observations have been made of pressure changes in a tributary free venous segment isolated between two occlusive pneumatic tourniquets (340 mm Hg). Venous catheterization from a puncture site distal to the tourniquets allowed the catheter tip to lie within the intact isolated venous segment. With the subject at rest, segment pressure remains stable for at least 5-7 min. Under these conditions a constant volume is maintained in the segment so that only reflex venous constriction produces an increase in pressure. Such constriction was consistently observed following deep inspiration, the Valsalva maneuver, immersion of the contralateral extremity in ice water and tilt from supine to upright position. Five subjects were studied during 15-sec exposure to 3 g (positive) on the human centrifuge. Pressure in the segment increased in every instance beginning 1-10 sec after the onset of acceleration, reached a peak after 10-30 sec and slowly returned to the original level during the subsequent 20-45 sec. The group mean maximum pressure increase was 12.5±4.8 mm Hg. Reflex venous constriction occurs during positive acceleration, presumably contributing to circulatory compensation. The functional significance of this reflex remains to be determined.

347. EFFECT OF ELEVATED VENOUS PRESSURES UPON BLOOD FLOW THROUGH THE DOG HIND LEG. Matthew N. Levy. Dept. of Physiology, Albany Med. College, Albany, N. Y.

Pressure-flow relationships obtained by varying the arterial pressure to the vascular bed of the dog's hind leg were compared with those obtained by varying the venous pressure. Reduction of the arteriovenous pressure gradient by decreasing arterial pressure produced a disproportionately greater diminution of flow. Graphs of pressure against flow are consistently curvilinear and convex to the pressure axis. On the other hand, when the pressure gradient was reduced by elevation of venous pressure, a reduction in flow was elicited which was not quite proportionate to the diminution in pressure gradient. In this case, elevation of venous pressure resulted in a very slight reduction of the calculated resistance to blood flow. Graphs of flow as a function of pressure gradient are virtually rectilinear, and extensions of such plots tend to intersect the flow axis at a very low, positive value. The pressure-flow data obtained by varying arterial pressure are interpreted as indicating passive changes in dimensions of high-resistance vessels due to changes in transmural pressure. The rectilinear plots observed with alterations in venous pressure suggest that elevations of venous pressure do not directly distend these high-resistance elements.

348. COMPARISON OF TISSUE AND PLASMA THROMBOPLASTIC ACTIVITIES. Jessica H. Lewis and Paul Didisheim.* Dept. of Medicine, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

A comparison was made of recalcification times of plasmas from patients suffering from various coagulation disorders, when saline, tissue thromboplastin or plasma thromboplastin was added. Plasma thromboplastin was prepared in the usual fashion, employing a mixture of normal serum, platelets, $BaSO_4$-treated plasma and calcium chloride. In the presence of saline, all of the tested plasmas had longer recalcification times than did normal plasma. Recalcification times in the presence of tissue thromboplastin were normal in AHF, PTC, PTA, Hageman factor and platelet deficiencies but prolonged in hypoproaccelerinemia, hypoproconvertinemia (Stuart), combined deficiencies associated with Dicumarol intoxication, vitamin K deficiency and severe liver disease and in normal heparinized plasma. Recalcification times in the presence of plasma thromboplastin were normal in AHF, PTC, PTA, Hageman factor, proaccelerin and proconvertin deficiencies, but were prolonged in the combined deficiencies in which prothrombin was greatly reduced, and in normal heparinized plasma. Thus striking differences in tissue and plasma thromboplastin recalcification times were seen in proaccelerin and proconvertin deficiencies. Almost all of the plasma thromboplastin activity could be sedimented at 20,000 g, and, when resuspended, this material could still normalize recalcification in these 2 deficiencies. These studies suggest that the plasma thromboplastin complex directly converts prothrombin to thrombin in the presence of calcium and that it may be inhibited by heparin.

349. TRUE PRIMARY EMOTIONAL STATE OF ANXIETY-TERROR-PANIC IN CONTRAST TO A "SHAM" EMOTION OR "PSEUDO-AFFECTIVE" STATE EVOKED BY STIMULATION OF HYPOTHALAMUS. John C. Lilly. Natl. Inst. of Mental Health, Bethesda, Md.

Ranson and Magoun, Bard and Masserman, among others, have maintained that activities and states occurring during heightened neuronal activity in specific zones in the hypothalamus are not "true" emotions; these states are merely signs of stimulation of the "peripheral motor mechanism for emotional expression"; and there is no true subjective experience corresponding to the clinical picture. From the following experiments, this point of view seems to us to be factually incorrect. In the walls of the 3rd ventricle above the stalk of the pituitary we have found a region, the stimulation of which evokes a clinical picture of anxiety-terror-panic in the unanesthetized monkey. During this state the pupils and palpebral fissures are dilated, the whole body shakes nonsynchronously; the directed movements are those of escape in an older monkey and those of holding on to the mother in a young one; if a non-food, potentially damaging object is brought within the visual field near enough to bite, the monkey reaches with his head and jaws and bites the object repetitively, so strongly that teeth are broken out of the jaw; this biting is continued until either the object is removed or the stimulation stopped. There is no evidence of pain stopping the biting during the stimulation; following the train, behavior connected with pain in the gums is seen (licking, refusal of food, etc.). We succeeded in training the monkey to switch off trains of stimuli at levels of current 1/5 to 1/2 the levels at which the clinical signs of anxiety appeared. In one monkey this habit was not extinguishable with a 12-day gap in training, whereas that for an extremely painful stimulus in the trigeminal nucleus extinguished within this period, and required re-training. Apparently this state is more "unpleasant" to the monkey than even severe pain.

350. EFFECT OF CERVICAL VAGOTOMY ON RESPIRATORY FLOW PATTERNS. Thomas P. K. Lim* and Fred S. Grodins. Depts. of Physiology, Lovelace Fndn., Albuquerque, N. M., and Northwestern Univ. Med. School, Chicago, Ill.

The effect of cervical vagotomy on pulmonary ventilation, respiratory frequency, flow rates and phase durations was studied in barbitalized dogs. In 16 animals, average control values were 3.23 l/min for ventilation, 15.1 cpm for respiratory frequency, 223 cc for tidal volume, and 85 cc/min for oxygen consumption. None of these was significantly changed by unilateral vagotomy, but following bilateral vagotomy, ventilation decreased to 2.74 l/min, respiratory frequency fell to 8.4 cpm, and tidal volume rose to 341 cc. All these changes were statistically significant. In 11 of the animals, flow patterns were recorded with a pneumotachograph. Average control values were: inspiratory maximum flow rate 31.8 l/min, inspiratory flow duration 0.9 sec, expiratory maximum flow rate 48.5 l/min, expiratory flow duration 1.1 sec, end-expiratory pause 2.2 sec. None of the animals showed end-inspiratory pauses. After unilateral vegotomy, the only significant changes were a 14% reduction in inspiratory maximum flow rate, and a 30% increase in inspiratory flow duration. Following bilateral vagotomy, inspiratory maximum flow rate was further reduced (-26% from control), inspiratory flow duration was greatly increased (+200%), and expiratory maximum flow rate significantly increased (+36%). In addition, short end-inspiratory pauses appeared in 5 animals. Neither expiratory flow duration nor the end-expiratory pause was significantly altered by vagotomy. (Supported in part by research grant H-1626-C4 from the Natl. Heart Inst., PHS.)

351. CEPHALIC PHASE OF GASTRIC SECRETION. T. M. Lin and R. S. Alphin.* Eli Lilly Research Labs., Indianapolis, Ind.

An attempt is made to evaluate the existence and significance of the cephalic phase of gastric secretion in the rat. Eight healthy male rats, weighing 350 gm, were used in this study. A chronic gastric fistula was fixed into the stomach several days or months before the experiments started. The day before the experiment, the esophagus of the rat was carefully freed from the vagi and the surrounding tissue, cut and anchored at both of the cut ends to the exterior of the neck. In the succeeding days, after the collection of the control gastric secretion for several hours, a small amount of finely ground PARD was fed to these animals. The volume, free acid and total acid of the sham-feeding periods were compared with the samples collected during the control periods before and after sham-feeding. Twenty-eight tests have been done, 14 of which revealed definite increase in the total volume, free acid and total acid output. In the presence of a "residual intestinal phase" resulting from previous feedings, the effect of sham-feeding on volume and acid output is overshadowed. This accounts for the failure to demonstrate the cephalic phase in half of the cases. The impression is gained that there is definitely a cephalic phase of gastric secretion in rats, and that this phase does not play an important role during physiological digestion for the gastric and intestinal phases of gastric secretion far exceed the cephalic phase in magnitude.

352. FIXED CHARGE HYPOTHESIS FOR A KEY MECHANISM IN CHANGE OF Na-K-PERMEABILITY DURING EXCITATION. Gilbert Ling. Eastern Pennsylvania Psychiatric Inst., Philadelphia, and Dept. of Psychiatry, Univ. of Illinois, Chicago.

A complete theoretical treatment of the Fixed Charge Hypothesis has been made in which it was taken into account: 1) all molecular forces (Coulombic and van der Waal's) between the interacting ions and dipoles; 2) the specific role of the fixed anionic groups. This treatment is based on known force constants and involves no use of values of hydrated ionic radii. It provides the quantitative solution for the remarkable rule governing the inversion of the lyotropic series recently discovered by Rudin, Eisenmann and Casby, who made the simple assumption that with increasing negative field strength the lesser hydrated cations will shed their water of hydration before the more strongly hydrated ones in an orderly and predictable fashion. Of particular interest in the interactions between fixed anions and alkali metal cations are those between protein (or lipo-protein) carboxyl groups and K, Na. With the anionic atomic dimension and polarizability thus defined, the relative magnitude of the total interaction energy between $Na^+ - H_2O - O^- R$ and $Na^+ - O^- R$ systems and between $K^+ - H_2O - O^- R$ and $K^+ - O^- R$ systems reverses itself in consequence of a small shift of the "center of gravity" of the excess electron on the carboxyl oxygen. This, in turn, changes the system of fixed charges from preferring K^+ to preferring Na^+. Supported by work on such fixed charge systems as exchange resins, connective tissues, frog muscles

the following hypothesis was made: a key mechanism in the change of high surface K^+ permeability to high Na^+ permeability in excitation is suggested to originate from such a shift of the excess electron, brought on by a change of interaction between other fixed charges located at a distance and some strategically important particles (removal of Ca, ATP?). The "charge effect" and "induction effect" thus produced would be transmitted to the carboxyl group in question along the polypepetide chains with their charge bearing side-chains; hydrogen bonds would transmit such effect intermolecularly. It is postulated that similar mechanisms underlie transmission of contraction in muscle. (Supported partly by Muscular Dystrophy Assoc.)

353. MEASUREMENT OF RATE OF DISTENSION OF HUMAN COLON. Martin Lipkin and Marvin H. Sleisenger (intr. by F. Gudernatsch). Dept. of Medicine, Cornell Univ. Med. College, New York City.

An experimental procedure has been developed to facilitate the study of elastic and viscous properties of intestinal muscle in humans. Intubation of the sigmoid colon in normal subjects has been carried out using catheters and latex balloons. One end of the catheter was attached to a cylindrical chamber containing a radiopaque liquid, the latter being permitted to flow into the balloon. The balloon diameter has been made sufficiently large so that distension of the viscus is not altered by the elastic properties of the balloon. Measurement of the rate of distension was made with a rapid exposure x-ray camera. Pressures within the balloon have been measured with a polyethylene catheter and an electromanometer recording system. The diameter of phantom models was measured to ± 1 mm. Pressures may be recorded to ± 3 mm H_2O. For analysis of the data, the distension of the intestinal segment and the applied pressure have been plotted against time. The rates of change of diameter with respect to time and to applied pressures have been plotted. The fractional rate of change of diameter has also been plotted with respect to pressure, and resistance to distension expressed in dyne seconds per cm^2.

354. SPEED OF PROTEIN SYNTHESIS. Robert B. Loftfield (intr. by Joseph C. Aub). Huntington Mem. Hosp., Harvard Univ., and Massachusetts Gen. Hosp., Boston.

The synthesis of ferritin (Loftfield and Harris. J. Biol. Chem. 219:151, 1956), like the synthesis of several other mammalian and bacterial proteins, has been shown to proceed essentially directly from free amino acids without the significant utilization of preformed peptides or proteins. Although some radioactivity can be found in protein within seconds after the administration of radioactive amino acids, it is not clear how much time is required for the entire process of assembling some hundreds of amino acids into an identifiable protein. Twenty hours after stimulating ferritin synthesis by iron injection, we have injected rats with C-14 L-valine or C-14 L-leucine. At intervals from 30 sec-80 min, lobes of the liver were removed and radioactivity in total protein and in ferritin (isolated chemically and immunologically) was determined. All ferritin formed after the second min had the same specific activity as the intracellular free amino acid. Hence the total time required for the assembly of enough amino acids to have the physical and antigenic properties of ferritin is only 2 min. From this it follows that the average life of all intermediates and precursors must also be no more than 2 min.

355. INFLUENCE OF CHRONIC BILATERAL VAGOTOMY ON MORTALITY RATE FOLLOWING CORONARY OCCLUSION IN THE DOG. David M. Long, Gerard D. Kaiser, John E. Sudol, William C. Shoemaker and Raymond C. Truex (intr. by Joseph R. DiPalma). Depts. of Anatomy and Surgery, Hahnemann Med. College and Hosp., Philadelphia, Pa.

Although it is generally accepted that the vagal and sympathetic nerves influence coronary blood flow, the precise mechanism and magnitude of this effect is not clear. These nerve pathways also have been implicated in the production of pain and ventricular fibrillation following coronary occlusion and coronary insufficiency. Sympathectomy, pericoronary neurectomy and basilar denervation have been performed in attempts to eliminate the above effects of myocardial infarction. It has not been demonstrated whether the beneficial results reported are due to interruption of reflex mechanisms or improvement in the blood supply of the infarcted areas. The effect of chronic vagotomy alone has not been investigated. In the present study bilateral vagotomy was performed by sectioning of the left cervical vagal trunk and extirpation of the right cardiovagal branches in the thorax. Measures were taken to prevent regeneration. Not less than 4 wk following bilateral vagotomy, the anterior descending branch of the left coronary artery was ligated within 5 mm of its origin. The coronary vessels were found to be significantly dilated at the time of ligation. The mortality rate was 33% in 15 chronic vagotomy dogs. In 15 control dogs, the mortality rate was 67%. (Supported in part by PHS Grants C-2627 and H-2003 (R).)

356. SIMULTANEOUS RECORDING OF MOTOR ACTIVITY OF PYLORIC SPHINCTER AND PROXIMAL DUODENUM WITH A TWO-CHANNEL INDUCTOGRAPH. Hortense Loukes* and J. P. Quigley. Dept. of Physiology, Univ. of Tennessee, Memphis.

We have modified the inductograph designed by Brody and Quigley (J. Lab. & Clin. Med. 29:3, 1944) to study simultaneously the motor activity of the proximal duodenum and the pyloric sphincter in the fasting state and during gastric evacuation in the normal, unanesthetized dog. The inductograph consists of two channels: channel "A" operates at 5.8 kc and channel "B" operates at 45 kc. To eliminate interference between channels, channel "A" amplifier is sensitive only to 5.8 kc and channel "B" only to 45 kc. One pair of coils was placed on the pyloric sphincter and a second pair on the proximal duodenum approximately 2 cm distal to the pyloric sphincter. In the fasting state (15-20 hr postprandially) the duodenal activity usually consisted of low amplitude "tonus" waves of approximately 1-2 min duration. Respiratory movements appeared to induce smaller waves superimposed on these "tonus" waves. In the fasting state the pyloric sphincter usually contracted in a rhythmic manner 4-6 times/min. During gastric evacuation the pyloric sphincter and usually the duodenum contracted in a rhythmic manner at a rate of 5-6 times/min. The duodenal contractions usually consisted of 2-4 small waves superimposed on a large wave of approximately 8-12-sec duration; occasionally there was a single contraction of 4-7-sec duration. The contraction of the pyloric sphincter usually preceeded that of the duodenum whereas relaxation occurred in the reverse order. (Supported by PHS Grant no. RG-2752 (C6S.).)

357. TEMPERATURE, HEART RATE AND OXYGEN CONSUMPTION DURING ENTRANCE INTO

HIBERNATION. Charles P. Lyman. Dept. of Anatomy, Harvard Med. School, Boston, Mass.

After entering hibernation, individual woodchucks (Marmota monax) were fitted with indwelling thermocouples and electrodes and placed in a metabolism chamber. These procedures invariably wakened the animals, which returned to hibernation after varying lengths of time. Temperatures from various parts of the body and oxygen consumption were recorded continuously, while the heart rate was recorded every 15 min. When the animal started to hibernate, the heart rate and oxygen consumption invariably declined at least 10 min before any drop in body temperature occurred. During the slow decline in body temperature, the heart rate could vary as much as 100% at identical temperatures in the same animal entering hibernation at different times. During partial arousal, heart rate and oxygen consumption increased before any rise in body temperature. While entering hibernation, the heart was the warmest part of the body and the central portion of the peritoneal cavity was cooler. This contrasted with the cooling of a freshly killed animal in which the peritoneal cavity remained the warmest. In the supine, nembutalized woodchuck, the heart and peritoneal area cooled together, but if the nembutalized animal was curled in the hibernating position, the heart cooled more slowly. This indicates that the curled position of the animal entering hibernation inhibits the flow of warm blood to the posterior. (Supported by A. F. Contract AF 33(038) 18133.)

358. PHYTOTOXINS IN THE URINES OF CERTAIN DISEASES. David I. Macht. Div. of Physiology and Pharmacology, Labs. of Sinai Hosp., Inc., Baltimore, Md.

The author studied for many years the influence of blood sera 1% on growth of seedlings in plant physiological solutions. All normal and most diseases, including bacterial, exert only a slight inhibition on root growth of Lupinus albus seedlings. Exceptions are: sera of menstruation, leprosy, pemphigua, and psychoses which are very toxic for root growth of normally reared seedlings; and the sera of psoriasis, syphilis, and especially cancer which are toxic for vernalized seedlings only. Do these diseases yield toxins demonstrable in the urine? Studies on all constituents of normal urines were made phytopharmacologically and revealed that the only phytotoxic constituent for the seedlings was urea. As a consequence 0.3% of human urine is more toxic than 1% of blood serum. On eliminating urea with urease the toxicity of urine normally becomes much less than in the original specimen. This procedure permits elimination of the masking toxicity of urea and indicates the presence of other toxic substances. By this method it was found that the urines of menstruation, pemphigus, schizophrenics and manic-depressives are toxic for growth of normally reared seedlings, while the urines of psoriasis, syphilis and especially of patients with malignant tumors are all markedly phytotoxic for vernalized seedlings of Lupinus albus.

359. SWELLING OF MITOCHONDRIA PRODUCED BY CCl_4. Sasha Malamed, Marilyn Litteria and Richard O. Recknagel (intr. by J. M. Werle). Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

In isotonic solutions of sucrose, NaCl, KCl or Na_2SO_4 saturated with CCl_4, normal rat liver mitochondria swell as evidenced by a decrease in optical density. CCl_4-induced swelling appears different from that induced by inorganic phosphate (Hunter and Ford. J. Biol. Chem. 216:357, 1955) for neither EDTA nor ATP affords protection. Furthermore, swelling with CCl_4 is much faster than with phosphate. The question arose as to whether CCl_4 causes swelling by an osmotic mechanism or by a direct destruction of the mitochondrial membrane. CCl_4 may increase the permeability of the mitochondria so that sucrose enters more rapidly than intramitochondrial solutes leave, resulting in a net flow of water into the mitochondrion. If this is the case, then swelling may be expected to be progressively delayed in proportion to increases in the external sucrose concentration. The swelling rate with increasingly hypertonic concentrations of CCl_4-saturated sucrose did decrease proportionately with higher sugar concentrations, suggesting that CCl_4-induced swelling is an osmotic process. A delay in mitochondrial swelling was also observed with increasingly hypertonic external concentrations of erythritol (a penetrating substance) in the absence of CCl_4. Phosphate-induced swelling is also delayed by hypertonic sucrose. Although both CCl_4-induced and phosphate-induced swelling appear to be basically osmotic phenomena, the different properties cited above suggest different modes of action. (Supported by PHS Grant No. A-329 (C4).)

360. ACID SECRETORY RESPONSES FOLLOWING HISTAMINE STIMULATION AND RELATIONSHIP BETWEEN "MAXIMUM ACID OUTPUT" AND NUMBER OF PARIETAL CELLS. I. N. Marks,* S. A. Komarov and Harry Shay. Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.

Gastric secretion in response to graded doses of histamine, given subcutaneously at 10-min intervals, was obtained from whole stomachs in acute experiments on dogs under Numbutal anesthesia. Extragastric contamination was excluded. Rates of secretion were measured at 10-min intervals; free and total acidities and outputs on pooled 30-min specimens. Each dose was maintained until a steady rate of secretion of 30-60 min duration was reached, after which the dose was increased. The experiment was continued in this manner until further increase in histamine dosage failed to increase the secretory rate. Typical sigmoid log dose/response curves was obtained for the rates of secretion at plateau levels, the corresponding acid outputs and, in some experiments, the acid concentrations. The magnitude of the histamine dosages required to effect the maximal gastric secretory response, and the responses themselves, were within the ranges observed by Hanson, Grossman and Ivy (Am. J. Physiol. 153:243, 1958) in intact dogs and in dogs with vagally denervated pouches. The cell counting technique was similar to that described by Marks (Quart. J. Exp. Phys., in press). In the present study, the number of parietal cells corresponding to the secretion of 1 mEq HCl/hr under the conditions of maximal histamine stimulation was within the range of that found in humans undergoing partial gastrectomy for gastric ulcers and carcinoma (Marks, unpublished data).

361. FORMATION OF RIBOSE AND GLUCOSE IN RAT LIVER IN VIVO. Paul A. Marks and Philip Feigelson (intr. by John V. Taggart). Medicine and Biochemistry Depts., Columbia Univ., College of Physicians and Surgeons, New York City.

Studies by previous workers have established that ribose phosphate may be synthesized by either 1) the oxidative decarboxylation of hexose phosphate or 2) the series of reversible non-oxidative reactions catalyzed by transaldolase and transketolase. The possible importance of these alternative pathways in

the biosynthesis of rat liver ribonucleic acid (RNA) ribose, as well as their role in the formation of glycogen glucose, has been evaluated by determining the C^{14} incorporation into each carbon atom of these sugars, employing intraperitoneally administered $C^{14}O_2$ and glucose-2-C^{14} (G-2-C^{14}) as the tracer sources. Ribose and glucose were degraded by fermentation with Lactobacillus pentosus and Leuconostoc mesenteroides respectively. In studies with G-2-C^{14}, the distribution of C^{14} in terms of percentage of total activity in RNA ribose was: C1-21.7%; C2-75.6%; C3-2.7%; C4-0%; C5-0%; and in glycogen glucose: C1-16.3%; C2-61.2%; C3-4.3%; C4-5.5%; C5-6.4%; C6-5.3%. $C^{14}O_2$ administration resulted in the following labeling of RNA ribose: C1-17.4%; C2-33.1%; C3-44.2%; C4-3.9%; C5-1.1%; and of glycogen glucose: C1-1.8%; C2-3.8%; C3-45.5%; C4-46.4%; C5-1.1%; C6-1.5%. Liver pyridine nucleotide ribose isolated in the $C^{14}O_2$ experiments had a distribution of tracer similar to that of RNA ribose. These findings are compatible with a contribution of both 1 and 2 to ribose biosynthesis. The patterns of labeling in glucose and ribose indicate that there is a rapid in vivo equilibrium between pentose and hexose pools and that in addition to the Embden-Meyerhof pathway, pentose conversion to glucose via the transketolase reaction may play a role in the formation of the glucose of glycogen.

362. HUMAN URINARY STEROID EXCRETION AS MODIFIED BY DIET AND SODIUM CHLORIDE. S. F. Marotta and S. G. Stolpe (intr. by F. Sargent II). Dept. of Physiology, Univ. of Illinois, Urbana.

Six healthy young men were used to determine the effects of various diets and NaCl intake on the excretion of urinary 17-ketosteroids, dehydroisoandrosterone (DIA), total ketosteroids, 17,21 dihydroxy-20-ketosteroids (Porter-Silber and Gornall chromogens), and 3-ketone steroids. The experimental dietary regimens comprised: 1) starvation (0 Cal.), 2) carbohydrate diet (2000 Cal.), 3) basal diet (2100 Cal., 59% CHO, 13% protein, 28% fat), and, 4) customary diet (unlimited Cal.). Four subjects were maintained on all diets, with and without 10 gm NaCl; two controls were subsisted on customary diets. Water intake was unlimited at all times. Daily urine samples were analyzed for the above steroids before and after the 3 to 4-day experimental periods. During starvation, and to a lesser extent on the CHO diet, all subjects showed a marked decrease from the preperiod 17-KS and DIA values, whereas only slight variations occurred during the basal and customary diets. When NaCl was given during the starvation, CHO, and basal diet periods a marked increase in 17-KS and DIA resulted on the first 2 days of the experimental periods. Total ketosteroids remained relatively constant on all diets, with and without NaCl, except during starvation, which produced marked decreases. All regimens, with and without NaCl, produced an initial drop in 3-ketone steroids and a slight increase in Porter-Silber chromogens. The above evidence suggests that changes in adrenocortical function produced by diet can be altered by NaCl ingestion.

363. EFFECT OF BREATHING OXYGEN ON PULMONARY VASCULAR RESISTANCE IN PATIENTS WITH ACYANOTIC CONGENITAL HEART DISEASE. Hiram W. Marshall,* H. J. C. Swan and Earl H. Wood. Mayo Fndn. and Mayo Clinic, Rochester, Minn.

Blood flows were measured and pulmonary and systemic arterial pressures and arterial oxygen saturation recorded continuously during the change from breathing air to oxygen in 31 patients with ventricular septal defects. Changes in patients with large left-to-right shunts were directionally similar, irrespective of the degree of pulmonary hypertension, and independent of general anesthesia. Average pulmonary arterial pressure of 75/37 mm Hg decreased to 90/89% (±2) of these control values 3 min after the change from breathing air to oxygen. This decrease began within 15 sec, and attained 60% of the 3-min value at 35 sec, while arterial oxygen saturation stabilized at 100% at 53 (25-120) sec. After 7 min, pulmonary pressure was decreased to 86/86 (±3)%, while pulmonary flow increased 34 (±7)% above the control value of 6.3 (1.8-19.4) $l/min/m^2$; systemic flow of 4.1 (1.6-8.2) was decreased 9 (±4)%; and systemic arterial pressure unchanged. Average estimated total pulmonary vascular resistance of 970 (120-2520) dynes sec cm^{-5} was decreased 47 (±9)%, while systemic vascular resistance (1635 (790-3530)) was increased 22 (±7)%. Mean pulmonary wedge pressures were unchanged. Thus, when patients with ventricular septal defects breathe 99.6% oxygen, a decrease in pulmonary vascular resistance usually occurs, associated with an increase in pulmonary flow and a slight decrease in pulmonary arterial pressure. Systemic vascular resistance usually is increased and, consequently, also the left-to-right shunt.

364. EFFECTS OF LOW TEMPERATURES ON TRANSMEMBRANE POTENTIALS IN ISOLATED AURICLES OF RABBITS. Jean M. Marshall. Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, Md.

The auricles were perfused with modified, oxygenated Kreb's solution, pH 7.4. The temperature of the perfusate was kept stable within ±0.5°C. Potentials were recorded with a KCl-filled microelectrode, and auricular contractions with RCA transducer #5734. The pacemaker region was localized in the right auricle near the entry of the great veins. Potentials recorded from this area showed the characteristic feature of the pacemaker, namely the diastolic prepotential culminating in the rapid upstroke of the action potential. As the temperature falls from 30°C the following is observed: 1) in pacemaker cells the rise of the diastolic prepotential slows, the amplitudes of "resting" and of action potentials decrease while the duration of the latter increases. After all contractions and conducted potentials disappear pacemaker cells continue to discharge usually until their "resting" potentials diminish from about 65 mv (30°) to around 50 mv. On rewarming pacemaker responses reappear first. 2) In areas close to the pacemaker region, the "resting" potential was about 72 mv (30°). With cooling the "resting" and action potentials diminish. Eventually a break appears in the upstroke of the action potential which suggests that the latter arises from a smaller, non-conducted potential. With further cooling the action potential disappears leaving only the small potential, which is also abolished at "resting" potentials below 60 mv. 3) In regions distant from the pacemaker, no small potentials appear with cooling. The "resting" potential falls from 72 mv to about 55-58 mv, and the action potentials disappear abruptly.

365. PRESSURE-VOLUME MEASUREMENTS ON DOG BRONCHI. H. B. Martin* and D. F. Proctor. Dept. of Environmental Medicine, Johns Hopkins Univ., Baltimore, Md.

Pressure-volume studies on excised segments of trachea and bronchi from freshly killed dogs were

performed in a plethysmograph in which the entire system was filled with saline. The mean outside diameters of two sizes of bronchi studied were approximately 5 mm and 2 mm. The hydrostatic pressure across the bronchial wall was varied by a leveling bulb and the resulting volume change was measured by displacement of saline into an accurately-calibrated pipette. When a pressure change was made the volume continued to change for periods up to at least 30 min. All measurements were made as quickly as possible in order to approximate the dynamic conditions of breathing. Hysteresis was demonstrated in all studies. The rachea completely collapsed with a pressure difference of 80 cm of water and had a greater compliance below its resting volume (volume with 0 pressure difference across it) than above its resting point. All bronchi were collapsed almost completely by transmural pressures of 10-30 cm water and had greater compliances above their resting point. If it is assumed that pressure around the bronchus is approximated by intrapleural pressure these bronchi at end-expiration have a volume 1-1/2-2 times their resting volume. They are also on the steep portion of their pressure-volume curve so that small changes in transmural pressure will cause large-volume changes and hence significant changes in resistance to airflow.

366. ROLE OF CARBOHYDRATE METABOLISM IN FATTY ACID OXIDATION. E. J. Masoro and J. M. Felts.* Dept. of Physiology, Tufts Univ. School of Medicine, Boston, Mass.

Previous reports from this laboratory noted that exposing fasting rats to environmental temperatures of 0-2°C caused a severe depletion of liver carbohydrate. This low carbohydrate content was accompanied by a marked decrease in $C^{14}O_2$ formation from acetate-1-C^{14} and butyrate-1-C^{14} by liver slices. Acetate-1-C^{14} oxidation was restored toward normal by the addition of glucose or pyruvate to the incubation medium, and glucose affected butyrate oxidation similarly. In the present investigation a larger series of fatty acids have been studied in an attempt to clarify the general role that carbohydrate metabolism plays in fatty acid oxidation. $C^{14}O_2$ production from valerate-1-C^{14}, caproate-1-C^{14}, heptanoate-1-C^{14}, octanoate-1-C^{14} was markedly decreased in livers from "cold-fasted" rats. The addition of glucose to the medium markedly stimulated $C^{14}O_2$ formation from these acids but had only a slight stimulatory action on the oxidation of these compounds in livers from normal rats. Palmitate-1-C^{14}, oxidation was not depressed in "cold-fasted" livers. Although the addition of glucose to slices from normal rats reduced $C^{14}O_2$ yield from palmitate-1-C^{14}, it markedly stimulated $C^{14}O_2$ production in livers of "cold-fasted" rats. Lactate was found to stimulate acetate-1-C^{14} and palmitate-1-C^{14} oxidation in cold-fasted livers in a manner similar to glucose. However, succinate, although utilized by "cold-fasted" liver in amounts similar to lactate, failed to stimulate the oxidation of palmitate in these carbohydrate-depleted livers. Evidently carbohydrate metabolism is necessary for an optional rate of long chain fatty acid oxidation. Apparently this stimulation is not related to the "sparking action" of Krebs cycle intermediates.

367. DISAPPEARANCE OF I^{131} LABELED LYMPH TRIGLYCERIDES AND PHOSPHATIDES FROM BLOOD OF DOGS. Esther L. McCandless* and D. B. Zilversmit (intr. by J. A. Dye). Division of Physiology, Univ. of Tennessee, Memphis.

Labeled lymph was obtained by feeding I^{131} triolein to unanesthetized dogs with cannulated thoracic ducts. The stability of the I^{131} label in the fat molecule during absorption was demonstrated by nearly complete recovery of the administered fat as TCA-precipitable material and the virtual absence of acid-soluble iodine compounds in thoracic duct lymph. Labeled lymph was administered to recipient dogs to determine the rates of disappearance of lymph lipides from plasma. Before extraction, plasma from recipient dogs was dialyzed against 0.9% NaCl to remove iodide which appears during breakdown of iodinated fat, and which accounts for 80-90% of the radioactivity present in plasma 2 hr after injection. Triglycerides and phospholipides in a chloroform-methanol extract of dialyzed plasma were separated on a silicic acid column and counted. Lymph I^{131} triglycerides were found to disappear from the circulation rapidly, with an initial half-time of several minutes. Simultaneously in the same animals, I^{131} phospholipides were cleared more slowly, 10-40% of the injected dose remaining in the blood 1-2 hr after injection. These results are similar to those obtained by labeling the triglyceride and the phospholipide components of artificially prepared fat emulsions. (Am. J. Physiol. 183:642, 1955). (Supported by grant H-1238 of USPHS.)

368. EFFECT OF POSTERIOR LOBE EXTRACTS ON ACTH RELEASE. S. M. McCann. Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia.

The effects of Pitressin on ACTH release have been evaluated in rats with blockade of the pituitary-adrenal stress response produced by pharmacological agents or hypothalamic lesions, using adrenal ascorbic acid depletion as an index of ACTH release. Nembutal-morphine anesthesia blocked the response to unilateral adrenalectomy but did not prevent a response to Pitressin, 0.08 U, intravenously (i.v.) (all doses expressed/100 gm rat), intraperitoneal (i.p.) epinephrine (0.02 mg), or ACTH (1.7 mU, i.v.). Hydrocortisone acetate overdosage blocked the response to unilateral adrenalectomy, but ACTH release from 0.17 U Pitressin, i.v., persisted. Rats with hypothalamic lesions and severe, permanent, diabetes insipidus (> 200 ml water intake/day) gave no ascorbic acid depletion to unilateral adrenalectomy, histamine acid PO_4 (1 mg, i.v.), i.v. epinephrine (2 μg), or Pitocin (1.7 U, i.v.), but responded with ascorbic acid depletion to Pitressin (1.7 U, i.v.) and ACTH. Hypophysectomized rats gave no response to this larger dose of Pitressin. Rats with acute lesions, 48 hr post-lesion, and diabetes insipidus failed to respond to operation, i.v. histamine, i.p. epinephrine, or Substance P(0.8 mg, i.v.), but responded to ACTH and Pitressin (0.08 U, i.v.). Pitressin evoked ACTH release of similar magnitude in rats with acute lesions and those with pharmacological blockade. There was a correlation between pressor activity and ACTH releasing activity on testing Protopituitrin and chromatographic fractions of Pitressin (method of Guillemin and Hearn) in rats with acute lesions. The results are consistent with the hypothesis that vasopressin is the neurohumor causing the acute release of ACTH in stress.

369. MYELOGRAMS RELATING TO ANEMIA AND HEMATOPOIESIS FOLLOWING THERMAL INJURY IN RATS. Miles D. McCarthy and John Odell.* Dept. of Zoology, Pomona College, Claremont, Calif.

Femoral marrow changes in weight, numbers of erythrocytes and leukocytes were studied in control, bled and burned rats. Burned rats received 20 and 50% surface burns. All rats received 18% body weight

of fluid: 4% lactate, 14% sodium chloride-1.4%. Marrow weights increased with age in controls. Bled animals showed decrease at 24 hr, a sharp increase at 48 hr followed by progressive increase through 4 wk; 20% burned animals showed an increase at 24 hr, a depression through 1 wk, followed by increased values. Values in 50% burned animals were depressed at 48 hr, then rose. Erythrocytes fluctuated in controls. These depressed in bled group during first wk; then paralleled those of controls. In burned groups, levels declined in direct relation to area burned during first 2 wk. Levels increased after 2 wk, but remained less than those of controls or bled rats. Changes in mature and immature neutrophilic granulocytes well correlated with those of total leukocytes. Total leukocytes of controls increased slightly in 8 wk. Bled rats showed decrease at 48 hr, a marked increase through 2 wk followed by a return to values of controls. Burned animals showed depressed values directly related to area burned through 1 wk. Values in 20% burned returned to control level by 4 wk whereas values of 50% burned remained depressed at 4 wk. (Supported by Research and Development Div., Office of the Surgeon General, Contract DA-49-007-MD-571.)

370. ENERGY EXPENDITURE OF HEREDITARY OBESE-HYPERGLYCEMIC MICE AND LEAN SIBLINGS. Ruth McClintock (intr. by Nathan Lifson). Physiology Dept., Univ. of Minnesota, Minneapolis.

Values for the total energy expenditure of hereditary obese mice obtained using an isotope method for measuring total CO_2 output over 1- to 3-day periods (Lifson et al. J. Appl. Physiol. 7:704, 1955, and McClintock and Lifson, J. Biol. Chem., in press) did not agree with the energy balance proposed by another laboratory (Mayer. Science 117:504, 1953) in 2 important respects: 1) the isotope method yielded a value for the total energy expenditure of control mice only about one half that previously proposed, and 2) the total energy expenditure of the obese mice obtained by this method was greater, not less, than that of the lean siblings. Since the previously proposed balance was based in part on "basal" O_2 consumption measurements, a study was undertaken of the O_2 consumption and CO_2 output of obese and normal mice under 3 sets of conditions, using an open-circuit method: a) approximately hourly measurements with animals previously fasted and confined in a narrow tube ("basal" conditions); b) 24-hr measurements with animals fasted and confined and c) 24-hr measurements with animals unconfined and given chow and water ad lib. The energy expenditure was estimated from the gaseous exchange and the observed R.Q. The energy output of the obese mice was found to be greater than that of the lean siblings under all 3 conditions studied, and results for total energy expenditure thus far support the values obtained by the isotope method. (Aided by a contract between the Office of Naval Research, and the Univ. of Minnesota, NR 115-366.)

371. SOURCE OF THE DORSAL ROOT REFLEX. Grayson McCouch and George Austin. Depts. of Physiology and Neurosurgery, Univ. of Pennsylvania, Philadelphia.

Under oil at room temperature, a stimulus to the cat's dorsal column 5.5 mm above the L6 root entrance zone induces a cord potential and a dorsal root reflex. The latter consists of 3 waves with average latencies of the apparent start of each wave of 3.4, 6.4, and 9.6 msec and crest latencies of 5.1, 7.4, and 10.4 msec respectively. Cord potential latencies average for start of N_1b 0.9 msec and crest 2.1 msec. For N_2 corresponding values are 5.1 and 6.6. The interval between the apparent starts of N_2 and R_2 is 1.3 msec and that between their crests is 0.8 msec without subtracting conduction time (about 0.3 msec). N_2 and R_2 are equally depressed by repetitive stimulation, by inhibition from an adjacent or an opposite dorsal root or by asphyxia. N_1b and R_1 are more resistant to inhibition or asphyxia. R appears in the cord potential as a small negative elevation between N_1b and N_2. We regard N_1b as the sum of postsynaptic potentials and spikes of interneurons of the first order firing repetitively. We interpret N_2 as the summed responses of interneurons subliminally activated by afferent terminals and fired by interneuronal endings plus the late components of the dorsal root reflex. We regard N_1b as the source of R_1 and N_2 as that of R_2. The long latency of R_1 may reasonably be attributed to relative refractoriness of afferent terminals. (Supported by Public Health Grant B277(C2) and Office of Naval Research Grant N-ONR-1047(00).)

372. ENDOGENOUS ADH PRODUCTION AND HYDROCORTISONE RELEASE. Roger K. McDonald, Henry N. Wagner, Jr.* and Virginia K. Weise.* Natl. Inst. of Mental Health and Natl. Heart Inst., Bethesda, Md.

In normal human subjects, under various experimental conditions, endogenous ADH activity occurred without increase in plasma hydrocortisone concentration; conversely, a rise in plasma hydrocortisone concentration occurred without evidence of ADH activity. Endogenous ADH release produced in 6 normal subjects by 18 hr water deprivation was associated with a progressive decrease in plasma hydrocortisone concentration indistinguishable from that observed in these same subjects in the hydrated state. In 5 subjects, the intravenous infusion of 200-300 ml of 5% saline during a 30-min period was not associated with a rise in plasma hydrocortisone in any of the subjects. Of 5 subjects showing ADH release following the intravenous injection of nicotine, 2 showed no increase in the plasma hydrocortisone level. Immersion of the hand in ice water for periods of 2-6 min resulted in antidiuresis in 8 of 9 subjects. Two of these subjects showed no rise in plasma hydrocortisone. A rise in plasma hydrocortisone occurred in the subject who showed no ADH activity. Insulin-induced hypoglycemia was associated with an increase in plasma hydrocortisone in 4 of 6 subjects. In 2 of these 4 subjects, no ADH activity was present; 3 of 4 subjects given methacholine intramuscularly showed a rise in plasma hydrocortisone levels; 2 of the 3 subjects evidenced no ADH release. These data fail to support the concept that ADH release mediates the adrenocortical response to various types of stress.

373. OXYGEN UPTAKE OF NON-WORKING VENTRICLE. William P. McKeever,* Philip C. Canney* and Donald E. Gregg. Dept. of Cardiorespiratory Diseases, Walter Reed Army Med. Ctr., Washington, D. C.

To measure the metabolism of the non-working myocardium, cardiac work must be reduced to zero and back to the control level while the oxygen consumption of the ventricle is determined continuously. This has been accomplished in the anesthetized open-chest dog by perfusing and measuring left coronary artery inflow under constant pressure, and draining externally coronary sinus blood. Portions of the coronary arterial and sinus blood streams were diverted through two photo-electric recording

densitometers to detect changes in oxygen saturation. Thus a continuous record of cardiac oxygen usage was obtained before and after stoppage of ventricular work. External cardiac work was reduced to zero by exsanguination through suction, and by cardiac arrest through vagal stimulation or intra-coronary infusion of potassium chloride. Immediately following vagal stoppage of the heart, its oxygen uptake increased above that in the beating heart and then within 10-15 sec fell to a lower level where it was maintained. In 14 hearts the latter value averaged 2.5 cc oxygen/100 gm myocardium/min. Expressed as a percentage of prior working levels the range was 16-32%. In 8 potassium stopped hearts and 9 bled hearts, the oxygen uptake averaged 2.2 cc and 2.1 cc/100 gm myocardium/min, respectively. Oxygen values obtained with the 3 methods in the same experiment were quite close. The significance of these findings relative to the oxygen debt and efficiency of the heart will be discussed.

374. A BIOASSAY OF THYROTROPIN. J. M. McKenzie (intr. by E. B. Astwood). Dept. of Medicine, Tufts Univ. Med. School and New England Ctr. Hosp., Boston, Mass.

Adapted from the guinea-pig method of Adams and Purves (Endocrinology 57:17, 1955) a sensitive bioassay of thyrotropin was developed in mice. It involved the measurement of what is probably the most specific effect of thyrotropin, viz. the discharge of hormone from the thyroid gland. Iodine in the thyroid glands of mice fed a low-iodine diet was labeled with I^{131} and endogenous thyrotropin secretion was suppressed by simultaneous injection of 10 μg thyroxine and feeding of .066% thyroid powder. Three days were allowed for the excretion of radioactive iodide. Then the radioactivity in 0.1 ml specimens of blood was measured before and 2 hr after the intravenous injection of thyrotropin dissolved in 0.2 ml saline. For 3 or 4 consecutive days groups of 6 mice were given varying doses of thyrotropin (U.S.P. Thyrotropin Reference Standard) or the substance being assayed. Control studies demonstrated that the injection of saline had no effect on the radioactivity of blood. The mean of 55 observations gave a result of 97% of the pre-injection activity, 2-3 hr after an injection of saline. Detection of 0.1 international milli-unit (IMU) was possible by this method, and a linear log-dose response curve was obtained between 0.1 and 1.25 IMU. The error of the assay in this range, as measured by the ratio (λ) of the slope of this curve to the standard deviation of the response averaged 0.25.

375. METABOLIC RESPONSES OF ADAPTED RATS TO ACUTE STRESS. Roy B. Mefferd, Jr., Henry B. Hale, and Herman H. Martens (intr. by Harry F. Adler). Biochemical Inst., Univ. of Texas, Austin, and School of Aviation Medicine, USAF, Randolph, AFB, Texas.

Using urinary determinations to indirectly assess metabolic "cost," comparison was made of a) effects of acute exposure to low barometric pressure on rats adapted to cold or to heat, b) effects of cold or heat on altitude-adapted rats, c) effects of cold on heat-adapted rats and d) effects of heat on cold-adapted rats. Including control situations, a total of 16 combinations of factors were studied. For each combination a group of 20 male Sprague-Dawley rats was used, and 24-hr fasting urine specimens were analyzed for 5 electrolytes and 17 nitrogenous constituents, including amino acids. Adaptation was accomplished by exposures of 12 weeks' duration. From determinations made on adapted animals in their customary environments and under standard conditions (25°C and 750 mm Hg) it was possible to adjust the values obtained under experimental conditions and to make direct comparison of adapted and control rats' responses. While for certain constituents, adapted rats showed exaggerated responses there were also instances of the opposite type. Since the magnitude of many of the responses shown by adapted animals was similar to that of control animals, it does not appear that the over-all metabolic "cost" for adapted animals was greater than for normal controls.

376. EFFECT OF NEPHRECTOMY AND PARATHYROID FUNCTION ON CITRATE DISAPPEARANCE IN THE RAT. Roger B. Meintzer* and Smith Freeman. Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.

Through the use of C^{14}-labeled citrate, disappearance from the blood stream was studied in mature albino rats. Under the conditions of these experiments, citrate had a half-life of approximately 47 min. Nephrectomy increased this value significantly (to 80 min), while bilateral ureteral ligation was without effect. This is further evidence of the important role played by the kidney in metabolizing circulating citrate. Parathyroidectomy by electrocautery increased the half-life of circulating citrate to approximately 65 min. Combined nephrectomy and parathyroidectomy increased circulating half-life to about 148 min. The observation that injections of parathyroid extract or large oral doses of vitamin D could restore the citrate half-life of parathyroid-less animals to near normal is suggestive of a significant role of calcium in determining these observed values. The possibility that these 2 substances affect citrate metabolism through entirely different mechanisms has not been excluded, however.

377. EFFECTS OF HORMONES ON INDUCTION OF SKIN TUMORS IN MICE. Joseph Meites. Depts. of Physiology and Pharmacology, Michigan State Univ., East Lansing.

Preliminary experiments (Meites, Trainin and Berenblum, 1955-56) suggested that the production of skin tumors in mice by chemical carcinogens can be significantly altered by the time sequence in which various hormones are given. Research now in progress indicates that when mice are fed thyroxine, thiouracil or hydrocortisone for a 10-day period prior to a single painting over the back with 9,10-Dimethyl-1,2-Benzanthracene, thyroxine inhibits while thiouracil and hydrocortisone enhance the production of tumors. On the other hand, hydrocortisone inhibits tumor production when it is started at the same time or following a single painting with the same carcinogen. Experiments with other hormones are not yet completed. These results suggest that initial treatment with hormones may influence the ability of chemical carcinogens to convert normal into tumor-potential cells ("initiating action") in mouse skin, while hormones given at the same time or following administration of carcinogens may alter the conversion of these cells into actual tumors ("promoting action") as well as change their growth rate.

378. UTERINE BLOOD FLOW IN PREGNANT GOATS. James Metcalfe, William E. Huckabee, Harry Prystowsky and Donald H. Barron (intr. by J. S. Nicholas). Harvard and Boston Univs., Boston, Mass., Johns Hopkins, Baltimore, Md., and Yale Univ., New Haven, Conn.

The blood flow to any organ can be determined by

measuring the arteriovenous difference across that organ of a diffusible foreign substance and analysis of the organ for its foreign substance content at the end of the arteriovenous measurement. This principle has been used to measure uterine blood flow in 37 goats at various stages of pregnancy. Venous blood was sampled through a catheter placed in a uterine vein at laparotomy. Nitrous oxide was initially used as the diffusible foreign substance. This gas when administered in 15% concentration does not reach equilibrium with uterine tissues in 30 min. Tissue analyses must be done anaerobically; 4 amino-antipyrine was used later. Equilibrium for this substance between blood and tissues can be secured in less than 15 min. At equilibrium tissue and blood concentrations are identical. Tissue analysis is unnecessary if the blood level at equilibrium is determined and tissue weights are known. Uterine blood flows measured by these 2 substances show good agreement. Values tend to parallel the curve of fetal growth. Analysis of arterial and uterine venous blood for oxygen content permits calculation of the oxygen consumption of the uterus.

379. ATAXIA AND THE RUBROSPINAL TRACT. Fred A. Mettler and Fernando L. Orioli.* Dept. of Neurology, Columbia Univ., College of Physicians and Surgeons, New York City.

In previous studies the present authors investigated the possibility of producing cerebellar ataxia by section of one or both of the descending efferent elements of the superior cerebellar peduncle notably the descending limb of the brachium conjunctivum and therubrospinal tract. It was found that a fully developed cerebellar ataxia could not be produced by such procedures but it was observed that rubrospinal section evoked homolateral hypokinesia. In the present study we have been concerned with a determination of what occurs when the rubrospinal tract is cut after a fully-developed ataxia has been previously established. Monkeys were used for this work, the ataxia being produced by severance of the right brachium conjunctivum as this enters the brain stem just below the inferior colliculus. Between 3 and 4 wk later the right rubrospinal tract was destroyed in the retro-olivary region. Such a procedure may or may not temporarily aggravate either ataxia or tremor or both but it consistently results in the addition of a definite hypokinesis to the preexisting symptomatology.

380. HUMAN HEAT LOSS DURING EXERCISE AT VARIOUS ENVIRONMENTAL TEMPERATURES. D. Minard, C. Kitzinger* and T. H. Benzinger. Naval Med. Research Inst., Bethesda, Md.

A new ergometer to be described more fully later has been designed for use with the human gradient calorimeter. The subject inside the chamber raises and lowers a weighted platform suspended outside. Cables running over pulleys couple handles and pedals to the load. To prevent heat and air leakage the cables are shielded and emerge through pneumatic seals. Forces are transmitted to outside structures, thus relieving the chamber from strain. Heat absorbing mass is also largely external, thus minimizing thermal inertia. Frequency and amplitude of the platform excursions are recorded. These data together with total platform weight are used to calculate external work. Heat loss has been recorded before, during, and after standard 40-min exercises performed by each of 2 subjects at 5 calorimeter temperatures ranging from 29.5°-21.1°C. In each test the ergometer registered 23-24 kcal of external work. At 29.5°C evaporative heat loss rose steeply as work began, reaching a high plateau in 20 min. Radiative and convective heat loss contributed essentially nothing to the excess heat appearing during work and recovery. Total excess heat was 100 kcal. At 21.1°C evaporative heat loss during work rose only slightly, the major fraction of excess heat being radiative and convective. Total excess heat was only 24 kcal. Thus in a cool environment heat produced during work is largely retained in the body and may serve to restore heat to tissues cooled during the preceding rest period.

381. BIO-ELECTRIC STUDIES OF THE COCHLEA. George Misrahy, E. Shinabarger, K. Hildreth and W. Gannon (intr. by H. O. Parrack). Wright Air Development Ctr., Wright-Patterson AFB, Ohio.

In order to obtain information concerning the action potential, D.C. potential of the scala media and resistance of the basilar membrane microphonics, measurements were made in 150 guinea pigs under Dial Urethane anesthesia. By means of a pair of stimulating electrodes placed in the scala media and scala tympani of either the first or second turn together with pairs of recording electrodes placed in turns one, two and three it was found that square pulses would not spread beyond the one containing the stimulating electrodes. Destruction of the fourth, third and second turn of the cochlea did not affect the D.C. potential or microphonics (1000 cycles) as recorded from the scala media through the round window. By measuring the potential drop produced by a constant current across the basilar membrane it was found that the resistance of this membrane was of the order of 500 ohms at the round window, 3500 at the first turn and 1000-1500 at the second turn. Loud sounds produce a disappearance of the action potential, microphonics and D.C. potential. Just preceding or simultaneously with the drop in D.C. there was a drop in resistance of the basilar membrane. Recovery of the resistance preceded the recovery of the D.C. Breathing nitrogen first lowered the D.C. potential of the scala media then the resistance of the basilar membrane, and on returning the animal to air the D.C. potential was the first to recover.

382. EFFECT OF CARBONIC ANHYDRASE INHIBITION ON THE CEREBRAL CIRCULATION OF THE ANESTHETIZED DOG. John C. Mithoefer, Paul W. Mayer and Joseph F. Stocks (intr. by James Bordley III). Mary Imogene Bassett Hosp., Cooperstown, N. Y.

A carbonic anhydrase inhibitor (Diamox) produced a prompt rise in cerebrospinal fluid pressure when given intravenously to 15 anesthetized dogs. The quantity administered ranged from 10-50 mg/kg, amounts sufficient to produce complete inhibition of carbonic anhydrase activity as measured in vitro. The cerebrospinal fluid pressure (CSFP) rose to an average maximal level 145% above the control value within 8 min after injection, then fell exponentially with time but had not yet returned to the control value after 80 min. This rise in CSFP is the result of an associated, measured, increase in cerebral blood flow resulting from cerebral arterial vasodilatation. It occurred in the face of a fall in cardiac output and in the absence of a change in systemic pulse pressure. The increase in cerebral blood flow occurred in the presence of a falling alveolar P_{CO_2} resulting from a simultaneous increase in alveolar ventilation. The coexistence of cerebral vasodilatation and decreased arterial CO_2 tension is opposite to the usual relationship in which cerebral vasodilatation has been attributed to arterial hypercapnia. Evidence will be presented to show that although the

arterial P_{CO_2} is low, the venous P_{CO_2} is elevated, suggesting the latter as the cause of the cerebral vasodilatation. This difference between arterial and venous P_{CO_2} will be explained as a necessary result of the inhibition of carbonic anhydrase. These data will be used to point out that the Henderson-Hasselbalch equation becomes inapplicable under circumstances of disequilibrium between CO_2 and carbonic acid resulting from the inhibition of carbonic anhydrase.

383. PRESSURES IN MOUTH AND PHARYNX DURING SUCKING AND SWALLOWING. K. Miyakawa, J. Kersey and A. R. Vela (intr. by R. H. Alden). Div. of Physiology, Univ. of Tennessee, Memphis.

Pressures were recorded in human subjects from the mouth and pharynx by a modification of the method of Quigley and Brody (Am. J. Med. 8:1, 1952) at 1 cm steps for 15 cm from the incisor teeth into the pharynx. During normal deglutition, a small closed cavity develops in the anterior oral cavity and progresses into the pharynx within 1 sec. Distinctly different pressures were found at the 1-cm areas from which pressure was measured. At the initiation of deglutition, the primary pressure change was strongly negative (as great as - 300 cm H_2O) near the incisor teeth. Apparently this negative pressure resulted from depression of the mandible with a closed oral cavity. Such negative pressures should draw saliva from the salivary ducts. As the pressure wave moved posteriorly, it became less negative and between 6-7 cm from the teeth (at the beginning of the pharynx), the primary wave changed from negative to positive. As the progressing motor activity was being measured 7-15 cm from the teeth, it produced 3-5 rapid pressure changes varying from -10 to +40 cm H_2O. Deglutition occurred during any phase of respiration and respiration ceased during the passage of the deglutition wave. When water was repeatedly sucked through a tube and swallowed, the marked initial negative pressure changes in the oral cavity were greatly reduced in magnitude. They were replaced by waves of negative pressure sufficient to transfer water from the container into the mouth. (Supported by USPHS grant G2752.)

384. EFFECT OF CLOT RETRACTION ON ELECTRICAL RESISTANCE. George W. Molnar. Army Med. Research Lab., Ft. Knox, Ky.

Rosenthal and Tobias (J. Lab. & Clin. Med. 33:1110, 1948) found that whereas the electrical resistance of heparinized blood remained unchanged, the resistance of unheparinized blood showed a sharp rise beginning at the time the blood gelled. They postulated that the rise was due to retraction and that retraction started immediately with gelation. To test these hypotheses resistance measurements were made at 37°C on mixtures of 1) fibrinogen and thrombin, 2) fibrinogen, thrombin and platelets, 3) fibrinogen, thrombin and erythrocytes and 4) fibrinogen, thrombin, erythrocytes and platelets. Each was in 0.9% NaCl, pH 7.4, bicarbonate buffer. Fibrinogen, thrombin and the formed elements were of bovine origin. Only mixture 4 showed both retraction and a rise in resistance; the retraction became visible after some 50 min whereas the resistance rise started at about 4 min after the addition of thrombin; at about 4 min an adjacent tilted tube showed gelation. Mixture 2 retracted but did not show a rise in resistance. Mixtures 1 and 3 clotted but did not retract nor show a rise in resistance. Hence Rosenthal and Tobias' hypotheses, that retraction begins with gelation and that retraction causes the rise in resistance, are validated. The clot, however, must contain corpuscles; retraction causes corpuscles (poor conductors) to displace conducting fluid from the interelectrode space. Since retraction starts early at the time of gelation, the useful measure of platelet activity is not retraction time but the progress of retraction. This is best shown by the course of the resistance curve, not by the delayed separation of serum.

385. EXTRACTABLE HEPARIN LEVEL IN RAT TISSUES. Frank C. Monkhouse and Ruth Mackneson.* Dept. of Physiology, Univ. of Toronto, Toronto, Canada.

Much of the work on the study of mast cells and their relationship to the histamine and heparin content of tissues has been carried out on rats. There is, however, little available information on normal heparin levels in different rat tissues, or on the properties of rat heparin. Levels for extractable heparin have been determined in 6 different tissues of the rat. The method used for extraction was that outlined by Monkhouse (Can. J. Biochem. and Physiol. 34:757, 1956). Slight modifications in the method were required for extracting heparin from rat skin. The following levels expressed in units/100 gm of tissue have been obtained; in liver, heart and spleen 0.24-0.44, in the lung and kidney 1.37-2.11 and in skin 3.3-8.1. The large variation in the levels obtained from skin reflect early difficulties in extraction. In the later experiments the values were higher and showed much less variation. Rats given cortisone showed a decrease in the heparin level of skin by the 9th day of treatment. The level had returned to normal by the 15th day. When BZ-55 was injected as well as cortisone the level of heparin skin was decreased by the 9th day and was still low by the 15th day of treatment. BZ-55 alone did not effect the heparin level. The antithrombic, metachromatic and anticoagulant properties of rat heparin have been compared and measured in terms of commercial beef heparin.

386. SURVIVAL AND BODY COMPOSITION OF HYPOTHALAMIC OBESE RATS IN STARVATION. D. G. Montemurro* and J. A. F. Stevenson. Dept. of Physiology, Univ. of Western Ontario, London, Canada.

To what extent does excess fat lengthen survival in acute starvation? Normal adult female rats survived starvation with water ad lib. for 16.4 ± 1.3 days. Rats made obese by hypothalamic lesions survived 42.7 ± 1.8 days ($P < .001$). There was, however, a point of diminishing return; the very obese survived only a little longer than the obese. A good correlation (- .993 ± .003) existed between the proportionate rate of body weight loss (gm/day/100 gm initial body weight) and the logarithm of the days of survival. Starvation obliterated the differences in body composition between normal and obese rats that are apparent in the fed state. The body water, fat-free body weight, and body protein of the once obese rats were now slightly but significantly smaller than those of the controls. The tremendous store of fat not only supplied a source of energy for a longer survival but also allowed a greater use of body protein and survival to a slightly lower total body weight. The resting metabolic rates of normal and obese rats were not different in the fed state, and both fell gradually during starvation. The prolonged survival of the obese cannot be attributed to a lower metabolic rate. Survival time was not well correlated with initial body weight or metabolic rate alone. But when these latter 2 indices were combined to relate the rate of

energy utilization to the pre-starvation store of energy in the ratio Pre-starvation metabolic rate/pre-starvation body weight another good predictive correlation was obtained with survival time (-.945±.021). (Supported by grants from the Natl. Research Council and the Defence Research Board of Canada.)

387. PHOSPHATE LIBERATION BY GLYCERINATED MUSCLE DURING CONTRACTION AND RELAXATION. C. Moos* and L. Lorand. Chemistry Dept., Northwestern Univ., Evanston, Ill.

Several agents (e.g. low temperature, reagents which destroy SH groups, "high" adenosinetriphosphate (ATP) concentration, inorganic pyrophosphate) which cause relaxation of glycerinated muscle fibers are known also to inhibit their ATP-ase activity. A similar correlation seems to hold for the relaxation induced by the "Marsh-Bendall factor" (Bozler. J. Gen. Physiol. 37:53, 1953; Weber and Portzehl. Progr. Biophys. 4:60, 1954). These observations led to the inference (Weber and Portzehl, above ref.) that relaxation in muscle is brought about by a reduction in the rate of ATP splitting to a level below that necessary for producing contraction. We, therefore, undertook to examine whether the relaxation induced by phosphoenolpyruvate (PEP) (Lorand and Moos. Fed. Proc. 15:121, 1956) is likewise accompanied by a decrease in the rate of ATP splitting. While the tension of the fibers was continuously recorded, aliquots were withdrawn from the surrounding bath at frequent intervals for inorganic phosphate determination. The fibers were allowed to contract isometrically in 4 mM ATP, 0.1 M KCl and 4 mM $MgCl_2$, and made to relax by subsequent addition of PEP to a concentration of 10 mM. Although a drop of tension greater than 70% may occur upon adding PEP, the rate of inorganic phosphate liberation has never been observed to decrease; on the contrary, it is often appreciably augmented. (Aided by a grant from the Muscular Dystrophy Assocs. of America.)

388. ALTERATIONS IN FELINE BEHAVIOR FOLLOWING BILATERAL AMYGDALECTOMY. P. J. Morgane* and A. J. Kosman. Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.

This experiment was designed to study the effects of bilateral removal of the amygdaloid complex on feline behavior. A group of 9 mature cats were observed preoperatively for 3-5 wk with respect to general cage activity, reactivity to routine care, response to petting, degree of aggressive behavior induced by application of noxious stimuli and reaction to other animals. Aspiration of the pyriform lobes containing the amygdaloid nuclei was performed in 2 stages. Post-operatively, aside from a transitory hyperactivity and hypervocality, general behavior changes were absent. However, a persistent hyperphagia became apparent in all experimental animals and this group showed a greater weight gain in the post-operative period as compared to a control group. In order to evaluate this hyperphagia ad lib. feedings were initiated on all animals. Although there were no significant differences in the initial weights of the operates as compared to the controls, four months after initiation of ad lib. feedings the difference was highly significant ($P < 0.001$). The food intake in the experimental group was found to be significantly higher than in the control group ($P < 0.001$). Regression analysis showed the rate of weight gain to be much more significant in the operates as compared to the controls ($P < 0.001$).

389. RELATION OF THYROID ACTIVITY TO INCREASED METABOLISM INDUCED BY FAT DEFICIENCY. D. M. Morris,* T. C. Panos,* J. C. Finerty, R. L. Wall* and G. F. Klein.* North Texas State College, Denton, University of Texas Medical Branch, Galveston, and Univ. of Miami School of Medicine, Coral Gables, Fla.

Several criteria of thyroid activity were used to determine the effect of a fat-deficient diet on the thyroid in weanling rats. Two control groups receiving adequate fat were used: one receiving the same number of calories as the fat-deficient series, and another kept at the same body weight as the experimental group. An observed increased rate of oxygen consumption in the fat-deficient rats confirmed reports of previous investigators. Twenty-four-hr thyroidal I^{131} uptake, 72-hr thyroidal I^{131} decay and PBI^{131} were not significantly different in any of the groups, while thyroid weights were lower in the fat-free series. Higher conversion ratios and T/S ratios in the fat-deficient series led to the conclusion that there was a greater rate of excretion of inorganic I^{131} in the fat-free group. It was concluded that increased oxygen consumption observed as a part of the fat-deficiency syndrome in rats is not due to hyperthyroidism. (Supported in part by grant A-380 from Natl. Insts. of Health, PHS.)

390. BODY TEMPERATURES IN ABORIGINALS. Peter R. Morrison. Physiology and Zoology Depts., Univ. of Wisconsin, Madison.

Incidental to studying marsupial temperature regulation some measurements were made on Australian aboriginals. Although these are limited and not under ideal conditions they are interesting in view of these peoples' almost complete transition from their natural unclothed life. A dozen 15-22-year-old persons were studied at Haast's Bluff 150 miles west of Alice Springs. This group had been clothed for the previous 6 yr, a third of their life, but wore only shorts during these observations. These were made in December, mid-summer, but during the night there was a brisk wind and air temperatures below 19°C and sky temperatures below 4° were recorded. Skin temperature were taken on 9 areas with a Stoll-Hardy portable radiometer in the hour before midnight (an hour after retiring) and again at 5-6 a.m. just before sunrise. Mean night values for the most exposed group of 6 men ranged from 27.7—foot to 33.2—forehead. Morning values were 20.8 and 30.4 respectively. The mean exposed skin temperature fell from 30.5-27.2 and the oral temperature from 37.0-35.1. A group of 6 girls with greater coverage (1/3 vs 1/6) and a group of 4 men on a warmer night (23° minimum —cloudy) showed only 0.8° fall for oral and 1.0-1.8° fall for exposed surface temperatures. Evidence of adaptation to cold is not seen since the body temperature fell markedly under moderate stress, but a lower sensitivity which allowed the subjects to remain asleep despite the low body temperature is indicated.

391. ADIPSIA AS A PRIMARY EFFECT OF LATERAL HYPOTHALAMIC LESIONS IN RATS. S. D. Morrison* and J. Mayer. Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

Adult female rats were made aphagic by electrolytic lesions in the lateral hypothalamus, as described by Anand and Brobeck (Yale J. Biol. Med. 24:123, 1951). The lesions were large. They extended from just rostral to the posterior hypothalamic nucleus to just caudal to the supra-optic nucleus, and from just

ventral to the fornix to the base of the brain. Their centers were lateral to the fornix and the lesions touched the inner boundary of the pyramids. The supra-optic nuclei, ventro-medial hypothalamic nuclei and the fornix were undamaged. The rats were also completely adipsic. Daily loss of body weight of these animals was about 65% greater than that of rats subjected to pure starvation but the weight loss pattern was closely simulated by normal rats given access to food but completely derived of water. Some rats showed spontaneous recovery of easting 3-8 days postoperatively; in most of these animals recovery of water intake slightly preceded recovery of food intake. Fasting gastric contractions for 48 hr postoperatively showed no difference in these rats from the contractions in normals. Addition of water, saline or sugar solutions directly into the stomach produced degrees of inhibition of motility comparable with those in fasted normal animals.

392. EFFECTS OF SOME INTRACELLULAR COMPOUNDS ON SMOOTH MUSCLE. W. Glen Moss and George A. Tershakovec.* Depts. of Physiology and Biochemistry, Univ. of Miami School of Medicine and Howard Hughes Med. Inst., Miami, Fla.

Adenosine and its phosphorylated derivatives produce relaxation when added to the environment of isolated smooth muscle of both ileum and non-gravid uterus. The tonic length of the preparation is increased and rhythmic activity is stopped, although rhythmic contractions will resume in a few minutes unless very large doses are given. Phosphorylation of adenosine increases its potency approximately in proportion to the degree of phosphorylation except for yeast adenylic acid (isomer B) which seems to be very weak. These compounds antagonize the effects of acetylcholine on intestinal smooth muscle and of histamine on the non-gravid uterus. Phosphocreatine and sodium phosphate have very little effect on either preparation when given alone. When given with adenosine type compounds the response is modified.

393. EFFECT OF TEMPERATURE, pH, TONICITY AND GLUCOSE ON ERYTHROCYTE UPTAKE OF $Na_2Cr^{51}O_4$, IN VITRO. Philip L. Mossman and Franklin G. Ebaugh, Jr. (intr. by Roy Swan). Dept. of Physiology, Cornell Univ. Med. College, New York City.

Grey and Sterling have shown that 80-90% of $Cr^{51}O_4^=$ added to erythrocytes becomes firmly bound to hemoglobin after 1 hr exposure. Little is known about the dynamics of this reaction. When from 0.22-0.9 μg of radioactive $Na_2Cr^{51}O_4$ were added to 1 ml of whole blood at 22°C at pH 7.4, 33% is taken up by the erythrocyte at 10 min, 48% at 20, 57% at 30, and 80% at 60 min. A change in temperature from 0.5°-40°C caused a 1100% increase in uptake 20 min after exposure to $Cr^{51}O_4^=$. The uptake increased 300% as pH fell from 8.0-6.7 and thereafter decreased 33% as pH dropped to 5.9. The uptake increased 40% when the erythrocytes were placed in 0.60 gm % saline and decreased 60% in 2 gm % saline. A pH effect can still be demonstrated when the data for the effect of pH were corrected for the concomitant change in cell volume. Suspension of the red cells in 7.6 gm % glucose caused a 900% increase in $Cr^{51}O_4^=$ uptake 1 min after its addition to the glucose-erythrocyte mixture. CO, KCN, NaF and iodoacetic acid had no significant effect on the uptake of $Cr^{51}O_4^=$ by the erythrocyte. The above effects could not be accounted for by viscosity changes alone although the temperature effects were consistent with the reduction reaction $Cr^{-6} \longrightarrow Cr^{-3}$. The tonicity, glucose, and pH effects cannot be explained by this mechanism.

394. COMPARISON ON HELIUM AND OXYGEN METHODS FOR MEASURING RESIDUAL AIR. Hurley L. Motley and William J. Kuzman.* Cardio-Respiratory Lab., Univ. of Southern California School of Medicine, Los Angeles.

The helium closed circuit system consisted of a Cambridge meter attached to a Collins Benedict-Roth metabolism machine with a blower. The inside valves were removed and short extensions attached to improve mixing. A measured volume of helium was introduced, sufficient to give a meter reading of at least 11%, then a measured volume, approximately 2 liters of air and oxygen (50% O_2) was added and the second meter reading obtained. The patient was connected to the closed circuit and the meter reading obtained every 30 sec until equilibration occurred. The CO_2 was absorbed from the circuit and the oxygen uptake during equilibration was added to the unknown volume calculated from the meter reading at the time of equilibration and corrected to BSTP. The expiratory reserve was determined at the time of turning into the helium for each residual measurement using a double valve assembly and Respirometer. The oxygen open circuit method was performed first followed by the helium closed circuit in 80 patients with a variety of clinical ailments. The absolute volume of the residual air varied from 400-5500 ml, and the ratio of the residual air to total lung capacity varied from 14-74%. In all cases except four the residual percentage of total lung capacity calculated from the helium determination revealed a difference less than 5% from that obtained by the oxygen open circuit method (S.D. 2.23%). (Supported in part by Research Grant S-9 (C) USPHS, Natl. Insts. of Health.)

395. POTENTIATION OF POSTNEPHRECTOMY HYPERTENSION BY THE 3 - CARBON UNITS, ALANINE, PYRUVATE AND LACTATE. E. E. Muirhead, F. Jones* and B. Brooks.* Dept. of Pathology, Univ. of Texas Southwestern Med. School, Dallas.

The mean arterial pressure of dogs determined over 4-5 days was averaged as the control pressure. A given animal's values seldom varied over 5 mm Hg (all controls averaged 116 mm Hg). Average weight was 10 kg. Both kidneys were removed; the arterial pressure was taken twice daily for 4 days. A sustained elevation of 25 mm Hg or more is considered hypertension. Group 1: 10 dogs received 16 ml/kg daily of physiologic saline intravenously and a diet containing 15 gm CHO, 7.5 gm fat and 21.5 gm alanine (volume 50 ml); 9 dogs became hypertensive (BP +25 to +65). Group 2: 5 dogs were irrigated peritoneally with one liter of ECF-like fluid daily containing 2.5 gm sodium pyruvate; no diet was given; fluid balance was maintained; 3 dogs became hypertensive (BP +45 and +50). Ten dogs (given no diet) received intraperitoneally daily 150 ml distilled water containing 2.5 gm of sodium pyruvate (amount of sodium comparable to alanine group); 8 dogs became hypertensive (BP +33 to +53). Group 3: 12 dogs (given no diet) received intraperitoneally daily 150 ml of distilled water containing 2.6 gm of sodium lactate; 9 dogs became hypertensive (BP +27 to +45). Of 37 dogs 29 became hypertensive. Previously (Fed. Proc. 15: abst. 1708, 1956) of 36 dogs receiving dietary protein and a similar quantity of sodium, 28 became hypertensive, while of 21 dogs receiving a similar amount of sodium and no protein 5 became hypertensive.

396. STABLE SOLUTIONS OF TRYPSIN. Otto H. Müller and Itsuro Yamanouchi.* Dept. of

Physiology, State Univ. of New York College of Medicine, Syracuse.

The selfdigestion of 20 mg % solutions of trypsin at 30°C in a variety of media was followed for at least 3 hr. The tryptic activity of samples taken every half hour was determined by a modification of Anson's hemoglobin method (J. Gen. Physiol. 22:79, 1938). In agreement with Kunitz and Northrop (J. Gen. Physiol. 17:591, 1934) it was found that in a M/15 phosphate buffer of pH 7.1 about 64% of the triptic activity is lost in 3 hr. Under the same conditions, but in a pH 7 bicarbonate buffer, trypsin lost 45% of its activity, hence the effect is not specific for phosphate buffers. In contrast to this, trypsin dissolved in water was found to be stable for at least 24 hr at 30°C. After this period a noticeable turbidity accompanies the loss in activity, which amounts to 11% in 48 hr, 51% in 72 hr, and 96% in 96 hr. If kept in a refrigerator, trypsin dissolved in water or in physiological saline solution retains its full activity for over a month. These findings are in contradiction to the results obtained with unbuffered solutions by Bier and Nord (Arch. Biochem. and Biophys. 33:320, 1951). However, the trypsin preparation used by these authors yielded an alkaline solution when dissolved in water, whereas ours (Armour's Tryptar) yields an acid solution. Additional experiments performed with buffers differing in total concentration of buffer as well as in pH lead us to the conclusion that our trypsin preparation reacts acid enough to prevent its self-digestion in unbuffered solutions. (Supported by a grant from the National Insts. of Health, PHS.)

397. REACTION TO HOT AND COLD NOXIOUS STIMULI IN NORMAL DOGS AND DOGS WITH HYPOTHALAMIC LESIONS. D. Murgatroyd, H. T. Hammel, H. Sharma and James Daniel Hardy (intr. by E. F. DuBois). Dept. of Physiology, Med. School, Univ. of Pennsylvania, Philadelphia.

Six mongrel dogs with lesions in the posterior hypothalamus and 2 normal controls were studied in regard to their reaction to nociceptive hot and cold stimulation. Reflex paw withdrawal upon immersion in a water bath was the response used to determine the dog's nociception of high and low temperatures. Measurements were made in terms of number of paw withdrawals per minute; observations were taken at temperatures of 47-48°C, 25-28°C, +1 to -1°C, -4 to -6°C, -7 to -8°C. Findings indicated that 1) all the dogs except one of the "probably totally impaired" dogs were nociceptive in some degree at 47-48°C. 2) For the dogs with hypothalamic impairment, their reaction in ice water would indicate that nociception due to cold was reduced compared to the normal dogs. 3) Lack of reaction in the normal dogs at 0°C might be taken as an indication that dogs can tolerate lower temperatures in their extremities with less discomfort than humans. 4) The nociceptive threshold for cold pain for the dog is somewhere between 0 and -4°C as compared to +18°C for the human hand.

398. CORPUS CALLOSUM AND INTERHEMISPHERIC COMMUNICATION: ENDURING MEMORY EFFECTS. Ronald E. Myers (intr. by Roger W. Sperry). Univ. of Chicago, Chicago, Ill., and California Inst. of Technology, Pasadena.

The corpus callosum subserves a highly-ordered intercommunication function between the hemispheres. Deficits following its destruction have been clearly seen only in the realm of "psychic" function. The meaning or signification of the experience of one hemisphere as defined by its input and integrations is in some manner transmitted in part or in whole to the other hemisphere through this commissure. The degree of definition and effectiveness of this intercommunication in terms of its ability to establish enduring mnemonic effects in the experientially "naive" hemisphere is not known. Transection of the corpus callosum in the chiasma-sectioned cat prior to monocular visual training completely precludes recall by the opposite untrained eye. In the present experiment the question of enduring mnemonic carryover to the "naive" hemisphere was investigated by corpus callosum-transection following, instead of preceding, the monocular visual training. The results of this experiment indicated good recall by the untrained eye of the simpler tasks but very poor to absent recall in the case of the more difficult tasks. This outcome is similar to that in which there was, instead of corpus callosum section, ablation of critical cortex from the "experienced" hemisphere following training. One may conclude that the intercommunication through the corpus callosum lacks the refinement and capability of the direct intrahemispheric integrating mechanism. Further, the loss of ability to perform the more difficult discrimination with the untrained eye following corpus callosum section indicates that the "experienced" hemisphere normally actively contributes to correct performance with the "naive" hemisphere through corpus callosum interaction.

399. CARDIAC DEPRESSOR EFFECTS OF CO_2 IN THE INTACT DOG AND HEART LUNG PREPARATION. G. G. Nahas and H. Mead Cavert (intr. by George Fahr). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

Anesthesia and respiratory arrest were induced in dogs by intravenous injection of 15 mg of Nembutal and 0.1 mg of succinyl choline chloride/kg. Mechanical ventilation was performed with room air and mixtures of 5-15% CO_2 in O_2 for periods of 10 min, in the intact animal before and following bilateral vagotomy, and after establishing a heart-lung preparation (HLP). Central venous and arterial pressures and the electrocardiogram were continually recorded. Arterial pH and, in the HLP, cardiac output were measured at regular intervals. In the intact dog, ventilation with 15% CO_2 (pH, 7.1) is consistently accompanied by bradycardia and cardiac arrhythmia; while pulse pressure increases, mean systemic pressure does not change. After bilateral vagotomy, a similar depression in pH is accompanied by a 20-30 mm Hg increase in mean systemic pressure and accentuation of TRAUBE-HERING waves. In the HLP ventilated with 10% CO_2 (pH, 7.2) bradycardia, fall in cardiac output, decreased arterial pressure, rise in central venous pressure and cardiac dilatation are observed. After 3 min on 15% CO_2, when arterial pH of the HLP approaches 7.1, output becomes negligible. This acidotic failure is rapidly reversible when the ventilating gas is reverted to 5% CO_2. Furthermore Nembutal added to the HLP accentuates CO_2 failure. These results may be interpreted as indicating that in the intact dog the negative inotropic effect of CO_2 on the myocardium is counteracted by stimulation of the adreno-sympathetic system.

400. EFFECT OF CEDILANID UPON THE EXCITABILITY OF THE INTACT DOG ATRIUM. L. H. Nahum and Dean Ashworth. Dept. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.

Electrodes of the type described by Delgado and Sikand were sewn upon the right and left atrium of the dog. Six to 12 hr post-operative when the animals were sufficiently recovered and breathing spontaneously

the excitability cycle of either or both atria was determined. Often this was repeated 2 hr later to determine the constancy of the graph. Then 0.8-1.6 mg cedilanid was injected intravenously and 1 hr later the excitability curves were again established. In all instances the electrocardiogram of lead 2 and left precordium showed a characteristic digitales effect upon the ventricles. At the same time electrograms from each atrium were recorded simultaneously with lead 2. After cedilanid the atrial excitability was only slightly different from the control record. There was an absolute refractory period, a relative refractory period with a smoothly declining slope, without dips or oscillations, and a uniform threshold that was raised from 0.1-0.3 M.A. Occasionally a supranormal period was present before cedilanid was injected, in which case it remained or disappeared after large doses of the drug. The total refractory period was not much prolonged except when the rate was slowed. It thus appeared that cedilanid introduced no new significant factors in the excitability of the intact atrium, the curve remaining much as it was prior to injection of the drug. The clinical significance of this finding will be discussed.

401. MALONATE METABOLISM BY MAMMALIAN TISSUES. Henry I. Nakada, Barbara B. Britton and Jack B. Wolfe (intr. by Douglas R. Drury). Scripps Clinic and Research Fndn., La Jolla, Calif., and Dept. of Med. Microbiology, Univ. of Southern California, School of Medicine, Los Angeles.

The in vitro metabolism of malonate has been studied using rat tissues. Slices of kidney, liver, heart and diaphragm were found to rapidly convert C^{14}-labeled malonate to $C^{14}O_2$, whereas spleen, brain, testes and lung were relatively inactive. The formation of acetoacetate from malonate by rat liver slices indicates that acetate is a product of malonate metabolism. Fractionation of rat kidney homogenates showed the complete system for malonate metabolism was associated with the mitochondria. Further studies demonstrated that coenzyme-A, ATP, Mg^{++} and glutathione were necessary for maximum activity. The formation of hydroxamate esters from malonate and the identification of malonylmonohydroxamate from the reaction mixture indicates that the probable mechanism of malonate degradation proceeds via malonyl-coenzyme-A, which is then decarboxylated to acetyl-coenzyme-A and CO_2.

402. THYROID AND GASTRIC SECRETION. E. S. Nasset, V. W. Logan,* M. L. Kelley, Jr.* and Mary Thomas.* Depts. of Physiology and Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Several dogs were provided with gastric pouches of the greater curvature. The feeding of lean beef or the subcutaneous injection of histamine were used to stimulate secretion of gastric juice. Volume and acidity of the juice were determined for 3 hr after initiation of the stimulus. Whole desiccated thyroid or extracts thereof were given by mouth and crystalline thyroxine was given by subcutaneous injection. Oxygen consumption was usually determined at the end of experimental periods and the intervening control periods. The freeding of enough desiccated thyroid to produce a moderate increase in O consumption (35-50%) usually results in a reduction of the secretory response to approximately half of the control value. Crystalline thyroxine generally does not duplicate the inhibition of gastric secretion which accompanies the feeding of whole desiccated thyroid.

403. ACTION OF CERTAIN ENZYMES ON THE FROG NODE OF RANVIER. Phillip G. Nelson (intr. by N. Kleitman). Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

By means of the bridge insulator technique of recording from single Ranvier nodes in isolated frog axons, the effects of several enzymes on electrical activity of frog nerve have been studied. Application of Ringer solution containing trypsin and chymotrypsin, 1 mg of each/cc, decreased the half-survival time (time at which 50% of the fibers were still functional) by about 38%. Phospholipase A, which splits off the first fatty acid from lecithin, reduced the half-survival time by about 86%. Phospholipase C, which splits off phosphoryl choline from lecithin, reduced the half-survival time by about 84%. It is tentatively concluded that phospholipid integrity is crucial for the maintenance of excitability in frog axons.

404. A VOLTAMETRIC DEVICE FOR CONTINUOUS MEASUREMENT OF OXYGEN PARTIAL PRESSURE. J. Ryan Neville (intr. by Richard W. Bancroft). Dept. of Physiology-Biophysics, School of Aviation Medicine, USAF, Randolph AFB, Texas.

The device utilizes a gold electrode as its working element, together with a tungsten reference electrode. The electrodes are fitted into a plastic holder and the face of the gold electrode (flush with the surface of the holder) is covered with an oxygen permeable membrane (approximately 1 ml thick). The unit is electrically connected as in conventional polarographic circuitry. Oxygen diffuses through the membrane and into the thin layer of electrolyte fluid between the electrode and the membrane. At a suitable voltage, the resulting electrolytic current is linearly related to the partial pressure of the oxygen in the surrounding gas. In tests run on gas mixtures (ranging from 0-100% oxygen and saturated with water vapor), the device has provided stable current output and given readings reproducible to within 1% oxygen. Day-to-day operation is more variable; however, the device is easily calibrated. Although dependent on the type of membrane used and the physical conditions affecting diffusion, the response time is fairly rapid: under optimum conditions, equilibrium is obtained within 3 sec. With agar added, the cell is practically free of interference from vibration and jarring.

405. SUBCORTICAL STIMULATION AS MOTIVATION AND AS SIGNAL FOR CONDITIONED RESPONSES. Harold C. Nielson,* Robert W. Doty and Lester T. Rutledge, Jr.* Dept. of Psychology, Univ. of Utah, Salt Lake City, and Dept. of Physiology, Univ. of Michigan, Ann Arbor.

Cats are trained to press a bar to receive food. Bipolar stimulation with 1-2 msec rectangular pulses is then administered in addition to food for 1.5 sec for each press. With frequencies of 50/sec stimulation of the caudate nucleus or of anterior area of median forebrain bundle with 1.0 mA. has no effect on pressing behavior, but septal or habenular stimulation at 0.6 mA. is immediately avoided. Caudate stimulation at 0.2 ma, 50/sec, is adequate as CS to establish foreleg flexion CRs. After such conditioning caudate stimulation at 0.3 ma, previously "neutral" at 1.0 ma, forestalls bar pressing despite extensive retraining. In other animals caudate stimulation at 300/sec, 1.0 ma, is rewarding as judged by bar responses up to 1600/half-hour if food is withheld. In such case prior feeding abolishes bar responses, whereas food intake is reduced 70% in food-deprived cats eating 40 min after such a period of self-administered caudate stimulation.

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The rewarding effects of stimulation seem best attained with frequencies and intensities of current considerably greater than necessary for perception as CS, and require rapid summation of several stimulations. If available on a schedule of one 1.5 sec-period every 5 sec rather than ad lib., the animal loses interest. Perhaps excessive or convulsive neural action is an important factor in such findings.

406. RENEWED GONOADOTROPHIC FUNCTION OF PITUITARY GRAFTS RE-TRANSPLANTED FROM KIDNEY TO MEDIAN EMINENCE. Miroslava B. Nikitovitch* and J. W. Everett. Dept. of Anatomy, Duke Univ., Durham, N. C.

The adenohypophysis loses most normal functions when its vascular link with the hypothalamus is permanently interrupted. Although several authors ascribe this primarily to loss of trophic influence of the hypothalamus, others suggest that it may primarily result from extensive loss of parenchyma caused by ischemia. The following experiment, however, demonstrates that such a damaged gland is capable, nevertheless, of renewed function once it has been replaced near the median eminence. In adult female rats, after parapharyngeal hypophysectomy, the pars distalis was autografted to a kidney. Two weeks later it was re-transplanted directly under the median eminence, using Harris' trans-temporal approach. In controls, in which the gland was poorly placed by design (or accident) or in which it was left in the kidney, the ovarian follicles and interstitium were consistently atrophic; the vaginas remained anestrous. When the graft was well placed under the median eminence, vaginal cycles returned 40-75 days later in 5 rats. One animal will serve as an example: cycling began at 41 days (July 23); copulation Sept. 1 was unfruitful; a successful pregnancy began Oct. 18. She could not deliver spontaneously and required caesarian section. She was later killed and perfused with India ink; appropriate tissues were studied histologically. The prominent sinusoids of the pituitary graft were in close relationship with capillaries of the median eminence. Basophiles and acidophiles were abundant, in clear contrast with control grafts. The "sella" retained an insignificant fragment histologically resembling pars tuberalis. In addition to stimulation of the ovaries there was significant restoration of adrenals and thyroids.

407. LYMPH CAPILLARIES IN THE FROG'S WEB. Yasuyosi Nisimaru and Hirosi Irisawa (intr. by F. R. Steggerda). Dept. of Physiology, Univ. of Hiroshima, Japan.

By repeated injections of India ink in Ringer solution, at low pressures, into the lymph sac of the frog's web, the cement substance of the lymph capillaries leading to the lymph sac becomes impregnated with India ink. Two hours after the injection the visualized lymphatic capillaries appear to be functioning normally and often remain outlined for nearly 24 hr for physiological observation. The average diameter of the lymph capillary compares well with that of the blood capillary ($18.5 \pm 5.8\mu$). The intraluminal pressure was 6.5 ± 0.64 mm of water. The dilation and contraction of the vessels is passive, with the extensibility of the wall at least 4 times that of the blood capillaries in the mesentary of the frog. The permeability of the injected capillaries to particles of increasing sizes was directly related to increases in intraluminal pressure when applied via the lymph sac. For example, Patent Blue dye escaped with 5 mm H_2O pressure, Congo Red with 20-50 mm and starch with 500 mm H_2O pressure. It was also observed that after injection of Patent Blue Ringer solution in the heart of the frog, the blood capillary bed was well visualized within 1 min, and that after 12 min the dye had left the blood capillaries and was found only in the lymph capillaries. After 27 min the dye could be found only in the lymph sac.

408. IN VITRO CHOLESTEROL SYNTHESIS IN BIOPSY-CONNECTIVE TISSUE OF THE ALBINO RAT. Nancy L. Noble and Robert J. Boucek (intr. by Stephen W. Gray). Howard Hughes Med. Inst. and Miami Heart Inst., Miami, Fla.

The in vitro synthesis of cholesterol by connective tissue isolated by the sponge-biopsy technique is comparable to that reported for aortic tissue of the rabbit and chicken and is approximately 1/200 that of the liver. The effect of the thyroid and of the sex hormones upon cholesterol synthesis in biopsy-connective tissue was studied in male and female albino rats. Slices of biopsy-connective tissue and liver from pretreated and control animals were incubated with 1-C^{14}-sodium acetate, and the radioactive digitonin-precipitable substance isolated and counted. Ablation of the thyroid with I^{131}, the feeding of desiccated thyroid to hypothyroid animals, the administration of thyroid stimulating hormone, desiccated thyroid or tretraiodothyroacetic acid to intact rats did not significantly alter the control value of acetate incorporation into the digitonide isolated from connective tissue-biopsy of either sex. Biopsy-connective tissue of female rats pretreated with relaxin or testosterone had significantly lower levels of radioactivity in the isolated digitonide. Pretreatment of male rats with relaxin resulted in a decrease in the labeling of the digitonide, while the hormones estrogen or progesterone effected significant increases. Cholesterol synthesis in liver tissue was decreased in the thyroid-deficient female rat and increased in the male pretreated with estrogen or tetraiodothyroacetic acid. Oophorectomy lowered cholesterol synthesis in connective tissue-biopsy while castration of the male rat was without effect when compared with the intact animal. (Supported in part by A.E.C. Contract No. AT-(40-1)-2070.)

409. EFFECTS OF REDUCED ALKALI RESERVE IN DOGS AND HUMANS. F. E. Noe and J. Nyboer (intr. by Daniel A. McGinty). Cardiovascular and Physiological Research, Harper Hosp., Detroit, Mich.

Extreme changes in pH in patients being prepared for cardiac surgery has drawn attention to the possibility of changes in respiratory compensation in cardiac subjects. Shephard (Br. Heart J. 17:153, 1955) noted some evidence of hyperventilation at rest and decreased carbon dioxide content of the arterial blood in most of 91 cases of congenital heart disease. Our own data and reports in the literature support these observations. An evaluation of the effects of lowered alkali reserve of acute and chronic nature is in progress. Normal dogs anesthetized with 30 mg/kg sodium pentobarbital and hyperventilated with an alternating positive-negative pressure respirator for periods as long as 10 hr suffered no consistent or sustained hypotension or other observed deleterious effects. However, dogs subjected to surgery using this combination of anesthesia and passive respiration showed greatly decreased carbon dioxide content of arterial blood and many of these developed intractible hypotension. Studies of human subjects indicate less compensatory activity in those with lowered alkali reserve. (Aided by grants and facilities made available by the American and the Michigan Heart Assocs, the McKesson Appliance Co., and the Cardiac Surgical Research Dept., Harper Hosp.)

410. FUNCTIONAL AND METABOLIC DEVELOPMENT OF THE MAMMALIAN RETINA. Werner K. Noell and Leonard H. Cohen.* Roswell Park Mem. Inst., Buffalo, N. Y.

The anatomical development of the sensory elements of the retina occurs post-natally in rabbits and coincides with the appearance of retinal function. This development was studied by the electroretinogram, the in vitro respiratory and glycolytic activities of the retina and the in vivo effects of poisons which specifically impair function and viability of the visual cells of adult animals. Simultaneously with the growth of the sensory elements and the appearance of the electroretinogram, especially of its b-wave component, the metabolism of the retina undergoes marked changes indicating the development of a system of very high respiratory capacity (Cohen, Fed. Proc., this issue). These differentiating processes occur with greatest rate during the age of approximately 12-18 days. They are followed by a second, slow phase of development during which the scotopic sensitivity of the electroretinogram increases to the adult level and during which the particular susceptibility of the visual cells to specific poisons (iodoacetate and high oxygen pressure) becomes manifest. This second phase continues up to or beyond the age of 60 days. It correlates with an increase in the retinal surface and in the volume of the sensory elements. It is not associated with significant changes of in vitro respiratory activity. (Supported by U. S. Public Health Grant B-812.)

411. INTRACELLULAR DISTRIBUTION OF SULFHYDRYL GROUPS IN LIVERS OF TUMOROUS AND NON-TUMOROUS RATS. Charles Norman, William G. Robertson and Ethel C. Montiegel (intr. by John C. Stickney). Dept. of Biology, West Virginia Univ., Morgantown.

The livers of rats inoculated with NK hepatoma and livers of non-tumorous rats were fractionated and the intracellular components analyzed for total nitrogen and sylfhydryl. Whole liver homogenates of tumor-bearing animals contained less total nitrogen than the controls ($P < 0.05$), total -SH/mg N did not differ statistically, implying a reduction in the -SH content of experimental liver. Aliquots of homogenate treated with protein precipitant gave non-protein -SH (NPSH) and protein-bound -SH (PSH) fractions. The NPSH/mg N was greater in the whole homogenate of tumor-bearing rats ($P < 0.05$), the PSH/mg N difference between groups was not statistically significant, suggesting no net movement of NPSH and a significant transfer of PSH from the liver of the tumorous animal. Livers of tumor-bearing rats exhibited a significant reduction in nuclear fraction nitrogen ($P < 0.05$) but no change in PSH/mg N, thus indicating a withdrawal of nucleoprotein from experimental liver. Total nitrogen and PSH/mg N in the mitochondrial fractions of experimental and control animals did not differ significantly (nuclear and mitochondrial fractions had no measurable NPSH). The supernatant fractions from experimental livers contained a significantly lower total nitrogen content ($P < 0.05$) and a significantly increased NPSH/mg N moiety ($P < 0.05$). The PSH/mg N differences between groups were not statistically significant. These data suggest that the tumor behaves as a nitrogen trap which probably promotes the preferential withdrawal from host liver of -SH labeled nuclear and cytoplasmic protein in contrast to an insignificant net transfer of NPSH and -SH enzymes attached to mitochondria. (Supported by the American Cancer Soc.)

412. EFFECT OF REDUCED PRESSURE ON THE RAT'S HEART. David W. Northup, J. Clifford Stickney and Edward J. Van Liere. Dept. of Physiology, West Virginia Univ. School of Medicine, Morgantown.

It was previously shown (Circulation Res. 4:217, 1956) that rats subjected to from 1-8 explosive decompressions to 32 mm Hg and killed 3-6 wk later showed no cardiac hypertrophy as a result of the cardiac dilatation produced by the procedure, but there was a possibility that a transitory hypertrophy may have occurred earlier. Therefore, 3 groups of at least 10 rats each were subjected to a single decompression to 32 mm Hg, and killed 2, 5, and 10 days later. The average heart weight-body weight ratio was 3% less than the controls in the 2-day group, 5% greater in the 5-day group, and 1-1/2% greater in the 10-day group. Since none of these changes was statistically significant, no cardia hypertrophy was demonstrated. The temporary cardiac dilatation produced by explosive decompression does not cause either temporary or permanent hypertrophy.

413. KORNER-SHILLINGFORD METHOD FOR MEASUREMENT OF VALVULAR INSUFFICIENCY. Paul Novack,* Robert C. Schlant,* Arthur O. Phinney, Jr.,* Florence W. Haynes and Lewis Dexter. Depts. of Medicine, Harvard Med. School and Peter Bent Brigham Hosp., Boston, Mass.

Seventy-five indicator dilution curves, using T-1824 or I^{131} tagged albumin, in patients without valvular regurgitation were analyzed by the method of Korner and Shillingford (K-S) (Clin. Science. 14: 553, 1955). Injection sites varied from a peripheral vein to left atrium (LA). Arterial samples were collected at 1- or 2-sec intervals. Aortic flow (AO) and central volume (CV) were calculated by the Hamilton method. As noted by K-S, the shape of a curve can be defined by the reciprocal of the downslope (1/s) and is dependent on AO and CV. Two regression equations were established relating these parameters for patients without regurgitation: 1) for CV > 1.00 l., $\log 1/s = 1.2335 - 0.0865\ AO + 1.396 \log CV$. Multiple correlation coefficient (R) = +0.87; the standard error of estimate of 1/s (S.E.) = 3.0. 2) For CV < 1.00 l., $\log 1/s = 1.4930 - 0.9068 \log AO + 0.3502 \log CV$. R = +0.80; S.E. = 2.8. (AO is in L/min and CV in liters.) In the presence of insufficiency, 1/s exceeds that predicted by these equations, and total ventricular output (TF) is calculated as suggested by K-S:

$$TF = AO \times \frac{\text{observed } 1/s}{\text{predicted } 1/s}$$

and regurgitant flow (RF) = TF - AO. Repeat determinations of RF from the same or different sites agreed relatively well, with a correlation (r) of +0.93 in 13 duplicate determinations from pulmonary artery (PA) injections; in 23 paired estimations from different sites (PA and LA) r = +0.92.

414. ORIENTATION IN ELEVEN FAMILIES OF BATS. Alvin Novick (intr. by D. R. Griffin). Biological Labs., Harvard Univ., Boston, Mass.

Seven genera of bats of the suborder Megachiroptera orient visually and give no evidence of acoustic orientation. The genus Rousettus orients acoustically in poor light but visually otherwise. The production of the sounds (controlled by the hypoglossal nerve) and the sounds themselves (largely low frequency) suggest a distinct origin of this system from the previously studied echolocation systems of the Microchiroptera.

Some 35 genera of Microchiroptera have been studied including the paleotropical families Hipposideridae, Rhinolophidae, Megadermidae, and Nycteridae. All of these echolocate. The characteristics of their ultrasonic pulses seem to fall into three major groups. The families Rhinolophidae and Hipposideridae produce sounds of relatively pure and constant frequency. The families Megadermidae, Nycteridae, Emballonuridae, Phyllostomidae, and Desmodontidae produce fundamental tones associated with one or more higher harmonics. The families Vespertilionidae, Molossidae, and Noctilionidae produce frequency modulated pulses. In addition to the frequency patterns, pulse duration, pulse repetition rate, intensity and directional beaming have been studied and some possible correlations noted with habits of flight and feeding as well as anatomical specializations such as nose leaves and elaborate external ears. (Supported in part by a contract between the Office of Naval Research and Harvard Univ., the USPHS, the Belgian American Educational Fndn. and the Sigma Xi-RESA Fund.)

415. CONSTANT MASS LOW FREQUENCY BALLISTOCARDIOGRAPHY. Jan Nyboer and Lucas Van Orden.* Depts. of Cardiovascular and Physiological Research, Harper Hosp., Detroit, Mich.

Records from normal subjects of increasing weight show measurable and visual differences in displacement, velocity and acceleration of a constant mass low-frequency ballistic bed which is subcritically damped and mounted as a pendulum. Marked differences in the head-foot plane were recorded with exercise, premature beats, breath holding, occlusion of the arms and legs and administration of nitroglycerine. The advantage of the method is that contrasting events in ballistics are quickly discerned by visual inspection as well as measurement. The clinical problems of valvular stenosis, insufficiency, myocardial insufficiency and hyperdynamic myocardium assert themselves graphically and more understandably. For a given subject the acceleration characteristics of the low-frequency ballistic bed are nearly equivalent to the displacement ballistic characteristics if measured directly from the shins by the same electromechanical transducers. This similarity is believed to be due to the body acting as an accelerometer to pulsatile movements of blood. We are of the opinion that what is called an "acceleration" record in the shin (DB-BCG) is in reality a double physical jerk initiated by the arteriovenous pulse, and is a fourth mathematical and electrical derivative of the displacement of the body effected by the pulse. (Aided by grants-in-aid and facilities from the New Hampshire, the Michigan and the American Heart Assocs., Dartmouth Med. School, May Hitchcock Hosp. & Clinic, Harper Hosp., Wayne Univ. Med. School, the Dow Corning Corp. and the General Motors Corp. Research Div.)

416. FUNCTIONAL REACTIVATION WITH FRUCTOSE OF THE HYPOTHERMIC HEART AFTER POTASSIUM ARREST. L. J. O'Brien,* F. W. Gonzalez* and M. M. Guest. Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.

Heart-lung preparations were used to study the effects of hypertonic fructose in the restoration of cardiac functional activity following KCl arrest in 19 mongrel dogs. Hypothermia was induced by lowering the temperature of the water bath surrounding the venous reservoir. When the desired temperature was reached (27-28°C), fibrillation was induced by faradic stimulation. Fibrillation was stopped by means of rapid retrograde injection of 7.5-20 ml 2% KCl in Ringer-Locke solution into the braciocephalic artery. Following a complete cardiac standstill of 1-16 min, 10-25 ml of 50% fructose in Ringer-Locke solution were added to the venous reservoir and cardiac massage was initiated. Slow cardiac massage (12-15/min) was continued until graphic registration of the heart beat was obtained (2-19 min). The first measurable cardiac output occurred from 2-26 min after the start of cardiac massage. There were no significant differences in cardiac outputs, mean blood pressures, peripheral resistances or venous pressures prior to fibrillation and following restoration of cardiac function. Serum potassium and reducing sugar concentrations following functional restoration of the cardiac activity exceeded the concentrations prior to fibrillation by 3.3 mEq/l. and 1365 mg% respectively. Serum sodium concentrations did not change significantly. Electrocardiographic changes in the restored hearts were usually absent except for slight alterations in the T wave. No gross indication of impairment in restored cardiac function was observed.

417. INFLUENCE OF DOSE OF TOTAL-BODY IRRADIATION ON SUCCESSFUL TRANSPLANTATION OF HOMOLOGOUS BONE MARROW IN RATS. T. T. Odell, Jr. and Barbara C. Caldwell (intr. by N. G. Anderson). Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

Establishment of persistent homotransplants of bone marrow in rats after 700-750 r x-rays was reported (Proc. Soc. Exp. Biol. & Med. 90:512). Rats having both alleles (DD) for D antigen on their erythrocytes were irradiated with 500, 300, 150, or 0 r of x-rays, and 18 hr later injected i.v. with bone marrow from rats having both alleles (CC) for C antigen on their erythrocytes. At intervals erythrocytes from the peripheral circulation were tested with anti-C serum for presence of CC cells. A few foreign cells made a transitory appearance in the 0 and 150 r groups. Two groups of 10 rats exposed to 300 r have been followed for 154 and 56 days, respectively. In one group 9 of 10 had some foreign erythrocytes at 15 days; by the 78th day one rat had essentially 100% donor type erythrocytes (also at 154 days), and regression occurred in the 4 other survivors. In the other group, 8 of 10 were positive at 14 days; at 56 days (last observation) 3 were nearly 100% positive and the remainder had regressed. Two groups of 10 rats exposed to 500 r have been followed for 146 and 14 days, respectively. In one group 4 of 9 were positive at 15 days; at 97 and 146 days one remained positive (82%) and the other 3 survivors had regressed. In the other group, 4 of 10 were positive at 14 days (last observation). The data indicate that persistent homotransplantation can occur after sub-lethal irradiation. However, unlike results observed after 700-750 r, the results after 300 r suggest that regression of the homotransplant occurs unless the bone marrow becomes 100% donor type.

418. ACCEPTANCE OF BLOOD BY THE VENTRICLES. Eric Ogden and Margaret Nishikawara.* Ohio State Univ., Columbus.

Elaborate experiments have been reported which were designed to explore the phasic changes in the ratio of ventricular inflow to intraventricular pressure. These have now been confirmed using a more direct method for measuring instantaneous inflow rate. During the period of greatest filling, the heart is more ready to accept blood (lower inflow impedance) in those experimental circumstances when the cardiac ejection is greatest. This relationship between the

readiness to accept blood and the ability to eject it is implicit, but not explicitly stated, in many experiments published during the past 40 yr or more. For instance, in whatever circumstances, experimental or clinical, digitalis increases the ejection per beat without a rise of venous pressure the heart is evidently accepting more blood from the venous system and, therefore, over the cycle as a whole, the heart offers a lower impedance to inflow. Since there is no inflow during systole, this lowering must be present during diastole. Similar reasoning applies when the heart fails with rising venous pressure. It is only when change of ejection is accompanied by a change in venous pressure in the same direction that a close phasic dynamic study is necessary to determine whether the ventricular muscle is altered as to its impedance to filling. Such a case is to be found in the increased impedance with cardiac acceleration previously reported. Changes in ejection are usually and perhaps always accompanied by a change in diastolic distensibility.

419. RENAL RESPONSE TO DIAMOX AT SEVERAL INTERVALS AFTER ADRENALECTOMY AND EFFECT OF DCG. Donald A. Olewine* and J. H. Perlmutt. Dept. of Physiology, Univ. of North Carolina School of Medicine, Chapel Hill.

In an attempt to determine the approximate time interval when certain renal defects develop after adrenalectomy, excretion of Na+, K+ and water was studied in adrenalectomized and sham-operated male rats at intervals of 3 days, 2, 3 and 4 wk post-operatively after an intraperitoneal injection of water (5% b. wt.) and after the same water load plus Diamox (25 mg/100 gm). Adrenalectomized animals were maintained on saline and intact animals drank tao water; 18 hr before the injections all animals were fasted but allowed fluid ad lib. The data show that the defective renal handling of K+ is evident by 3 days post-operative; although Diamox increased excretion of K+, the values were significantly less than for the controls indicating an impairment of distal tubular secretion of K+ after adrenalectomy. Impaired excretion of a water load was also evident by 3 days, but was not maximal until after that time; after Diamox, urine volume/100 gm showed a progressive decline indicating increased tubular reabsorption of water. Excretion of Na+ in excess of the sham-operated animals occurred at the 3-wk interval after water or Diamox. Desoxycorticosterone glucoside (0.5 and 2.5 mg), injected s.c. 1-1/2 hr before the injection of water and Diamox, increased the excretion of K+ over Diamox alone and caused some retention of Na+ with no appreciable change in urine volume. The data suggest that this sterioid exerts its effect by increasing the distal tubular secretion of K+ thus providing more K+ for exchange with Na+. (Supported by AMA and PHS grants.)

420. ESTIMATION OF WORK DONE ON THE LUNGS DURING BREATHING. Arthur B. Otis. Physiology Dept., Univ. of Florida College of Medicine, Gainesville.

During inspiration elastic work may be done on the lungs by 1) the inspiratory muscles and by 2) the elastic forces of the chest wall. During expiration elastic energy may be returned from the lungs to the chest wall. Some workers have attempted to calculate the elastic work done on the lungs from measurements of the difference between intraoesophageal pressures at instants of zero flow and of tidal volume. Such calculations are erroneous because they neglect the interaction between the lung and the chest wall. The correct basis for estimating the elastic work done on the lungs will be demonstrated by use of the pressure-volume diagram.

421. USE OF AEROSOLS TO MEASURE INTRA-PULMONARY MIXING. Edward D. Palmes, Bernard Altshuler and Leonard Yarmus (intr. by L. W. Eichna). Inst. of Industrial Medicine, New York Univ., New York City.

In the study of the effect of particle size on aerosol deposition in the human respiratory tract, a method was developed for simultaneous measurement of respiratory flow, aerosol concentration and CO_2 concentration. Uniformly sized aerosols of small diameter (0.14-3.2 μ) were employed. Particles in the middle of this size range have considerable stability in the supporting gas and correspondingly show minimum deposition. Such particles are exchanged between the tidal and reserve air and show moderate persistence in the reserve air. Thus it was found that when a subject began to breathe aerosol several breaths were required before a constant fraction of the particles was retained. When he was shifted back to clean air several breaths were required before the exhalations contained no particles. At the size of minimum retention (0.4 μ diameter, about 20% retention) it was possible to quantitate the effects of this phenomenon. The results can be used to estimate the extent of mixing of tidal air in the respiratory tract. Results obtained on 3 normal subjects will be discussed. (Aided by a grant from the Atomic Energy Comm. contract AT(30-1)-958.)

422. UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY THE FREE RADICAL, TETRAMETHYL-p-PHENYLENEDIAMINE. Jane Harting Park,* Blanche Pittman Meriwether,* C. R. Park and Leonard Spector.* Dept. of Physiology, Vanderbilt Univ., Nashville, Tenn., and Huntington Research Lab., Massachusetts Gen. Hosp., Boston.

Hamster liver mitochondria, incubated as previously described (Biochim. Biophysic. Acta, 22:403), oxidize the colorless tetramethyl-p-phenylenediamine to a free radical with a deep purple color. With beta hydroxybutyrate as substrate and glucose and hexokinase as the acceptor system, the purple tetramethyl-p-phenylenediamine (2×10^{-4} M) uncouples oxidative phosphorylation and lowers the control P/O ratio from 2.4-0.5. The uncoupling by tetramethyl-p-phenylenediamine is antagonized by magnesium. Increasing the magnesium concentration from 3.3×10^{-3} M to 1.3×10^{-2} M raises the P/O ratio from 0.5-2.2. Glutathione (5×10^{-3} M) and ethylene-diaminetetraacetate (1×10^{-2} M) also restore the phosphorylation in the uncoupled system as shown by P/O ratios of 2.0 and 2.1 respectively. Restoration of phosphorylation by these 3 compounds is always accompanied by a decrease in the concentration of the free radical form of the tetramethyl-p-phenylenediamine as indicated by either a complete or partial loss of the purple color. Uncoupling by the free radical has also been demonstrated with rat liver mitochondria. As with thyroxin, the range of magnesium concentration which would permit normal P/O ratios in the control vessels but not antagonize the uncoupling effect of the tetramethyl-p-phenylenediamine was found to be very narrow. The findings with tetramethyl-p-phenylenediamine and thyroxin are similar in that uncoupling by thyroxin (1×10^{-4} M) is also antagonized by magnesium, glutathione and ethylenediaminetetraacetate.

423. INDUCED VISCERAL HYPOTHERMIA IN ACUTE HEMORRHAGE AND HEMORRHAGIC

HYPOTENSIVE SHOCK. W. M. Parkins, S. J. LeBrie* and M. L. Coady.* Harrison Dept. of Surgical Research, Univ. of Pennsylvania School of Medicine, Philadelphia.

Visceral cooling (i.p. administration of 1-5°C saline), effective in prevention of irreversible ischemic shock induced by occlusion of the thoracic aorta, has been applied to 3 groups of bled dogs. 1) Acute bleeding (Bp of 30 mm/Hg) without replacement: the percentage of the blood volume removed was determined and increments in Bp, blood and plasma volume were followed and compared with normothermic controls. This method of hypothermia designed for rapid cooling (to 25°C) of the intestine did not interfere significantly with the spontaneous compensation of dogs bled 40-60% of their blood volume. All these animals survived. 2) Hemorrhagic hypotension with blood replacement: two hours at 30 mm/Hg in dogs under Nembutal anesthesia was fatal to all normothermic controls within 24 hr. Eighty percent of the hypothermic animals survived 24 hr and 40% survived beyond 72 hr. When the visceral cooling was delayed 15 min into the hypotensive period, 60% survived 24 hr whereas 20% survived more than 72 hr. 3) Hemorrhagic hypotension with occlusion of the thoracic aorta: 20% of normothermic controls without saline and 80% with i.p. saline (37°) survived an interval of 30 min of hemorrhage-aortic occlusion. No normothermic dog, with or without saline, survived 60 min of this stress followed by blood replacement. All hypothermic animals survived 30 min and 90% survived 60 min of this severe visceral ischemia. (Supported in part by Army Contract DA-49-007-MD-511.)

424. GROWTH STIMULATING ACTIONS OF LIVER PREPARATIONS. K. E. Paschkis, A. Cantarow and J. W. Goddard.* Div. of Endocrine and Cancer Research, Jefferson Med. College, Philadelphia, Pa.

We have previously reported that certain malignant tumors grow more rapidly in partially hepatectomized rats (Canc. Res. 15:579, 1955), and that treatment of intact tumor-bearing rats with certain liver preparations enhances growth of the tumors (Fed. Proc. 14:25, 1955). We have now studied the influence on growth and on mitotic activity in other tissues, of partial hepatectomy and of liver fractions which are active in enhancing tumor growth. A suspension of an active liver fraction was injected subcutaneously into rats; on the 10th day, colchicine was injected, and the animals were killed 8 hr later. A significant increase of mitosis was found in liver and in cornea, but not in skin (pinna) or kidney. Kidney growth was also investigated by studying the compensatory hypertrophy of the remaining kidney following unilateral nephrectomy. This type of growth is, in contradistinction to the growth phenomena described above, an hypertrophy, and not associated with cell divisions. The compensatory hypertrophy was greater in partially hepatectomized, heminephrectomized rats, than in those which had been subjected to unilateral nephrectomy only. It would appear that both the regenerating liver and the administration of liver preparations cause increased growth in some, but not in all tissues.

425. EFFECT OF PARTIAL CEREBRAL DESTRUCTION ON OPTOKINETIC NYSTAGMUS. Pedro Pasik,* Tauba Pasik* and Howard P. Krieger. Dept. of Neurology, Mount Sinai Hosp., New York City.

The location and extent of the lesion of the primate cerebrum which leads to defective optokinetic nystagmus (OKN) is a matter of debate. Lesions of both cortex and subcortex and lesions of both specific cortical foci and large parts of diverse regions of the cerebrum have been associated with defective OKN in man. The present study was an attempt to define the locus and size of cerebral destruction required to alter OKN in 15 M. mulatta. The following regions were aspirated uni- and bilaterally: 1) frontal eye field, 2) ventrolateral temporal cortex, 3) posterior parietal cortex, 4) occipital lobe, including the entire calcarine cortex and 5) these regions in various combinations. All animals were repeatedly examined neurologically and behaviorally both before and after each procedure. OKN was elicited with a stripped drum filling the entire visual field and was observed by recording changes in the corneo-retinal potential. The only lesion found to eliminate OKN was bilateral occipital lobectomy great enough to remove all responses to light except pupillary constriction and blinking. All other lesions had no effect upon OKN, even combined ipsilateral removal of the frontal and preoccipital eye fields and the occipital lobe. (Aided in part by USPHS Grant B-294.)

426. KINETICS AND ENERGETICS OF A NON-CARRIER MODEL FOR ACTIVE TRANSPORT. Clifford S. Patlak (intr. by Franklin C. McLean). Committee on Mathematical Biology, Univ. of Chicago, Chicago, Ill.

A class of active transport mechanisms, apparently not previously considered, is that of the "Maxwell Demon" to which energy is coupled. Such a mechanism is consistent with the second Law of Thermodynamics. A Maxwell Demon mechanism does not carry the particle across the membrane but instead alters the properties of either the membrane or the particles allowing these to cross by themselves. A specific example is the "gate-type Maxwell Demon" where the Demon, a fixed, spatially oriented molecule within the membrane, acts as a "gate" (a potential barrier to particle movement across the membrane), selectively "opening" and "closing" to allow a net transport in one direction. The Demon molecule first combines with a particle (for example) on its left side forming a complex. A series of coupled energetic reactions, forming new bonds and breaking old bonds, rearrange the complex so that there is an opening to the right and a closure to the left of the particle. Thus when the particle is released, it can only diffuse to the right. The Demon then returns to its original form and the cycle may repeat itself. An important property of this mechanism is that its width need not be more than a few Å, thus fitting within a thin membrane. The resultant kinetic equation of this mechanism cannot be distinguished from those of many carrier models. The above mechanism may have a very high efficiency.

427. NEUTROPHIL RELEASE FROM BONE MARROW. Harvey M. Patt, Mary A. Maloney,* Eugenia M. Jackson* and Robert L. Straube.* Argonne Natl. Lab., Lemont, Ill.

As part of a study of leucocyte regulation, blood from leucopenic x-irradiated dogs was shunted through the isolated pelvis and hind legs of normal animals. The irradiated dogs received 400 r (total body) 6 days prior to cross-circulation. Leucocyte antiserum was given during this interval to hasten marrow exhaustion. The neutrophil levels of blood flowing to and from the isolated extremities equilibrate within minutes. A rapid increase of neutrophils in blood from the perfused part follows, band cells and metamyelocytes constituting about 85% of the total after 30 min of perfusion. The number of

neutrophils released during the first hour, calculated from the mean venous-arterial difference and blood flow, ranged from 10-40 X 10^9 or approximately 2-5 times the detectable circulating mass before isolation of the extremities. Most of the cells contributed by the perfused part are apparently lost rapidly from the circulation; the traumata incidental to the procedure doubtless contribute to this. There is some indication of a preferential loss of segmented neutrophils. These data reveal the vast number of cells available for release from marrow, since the perfused area represents only about 25% of the body weight. The order of cell numbers is consistent with theoretical considerations. (Work performed under auspices of the U. S. Atomic Energy Comm.)

428. ELEVATION GRADIENT OF INTRATHORACIC PRESSURE. John L. Patterson, Jr., John J. Krueger* and Thomas H. Bain.* Dept. of Medicine, Med. College of Virginia, Richmond.

Intrathoracic pressure represents a basic element in the mechanics of breathing. Conventional technique requires the production of a degree of pneumothorax and precludes measurements in multiple areas. In the present studies a localized mobile "pneumothorax" was utilized. A 1.5 cc thin rubber balloon containing 0.5 cc air and attached to a 1.4 mm ID polyethylene tube was introduced into the intrapleural space through a tightly sealed trocar inserted either at the lung apex or in the third intercostal space and passed down the lateral lung surface to the base. Eleven dogs, anesthetized with sodium pentobarbital, were suspended in the upright position and pressure continuously recorded during step-wise elevation of the balloon's position. Despite some individual variation, the mean intrathoracic pressure fell linearly with distance from the lung base. The pressure gradient was 0.21 cm H_2O/cm elevation. In 2 dogs, the specific gravity of the lungs in the end tidal position averaged 0.22. Specific gravity of human lungs calculated from standard values for functional residual capacity and total lung weight was 0.21. It is concluded that intrathoracic pressure varies with elevation as would the pressure in a hypothetical fluid of the same specific gravity as the lungs. On this basis, intrathoracic pressure in man in the upright position should be 5 cm H_2O lower at the apex of the lung than at the base.

429. CONTRIBUTION OF CELLULAR ELEMENTS TO PRIMARY RESPONSE OF SOMATOSENSORY CORTEX. Harry D. Patton and Arnold L. Towe.* Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.

In chloralose-decamethonium anesthetized cats, primary response (somatosensory area I) to contralateral forepaw stimulation consists of early (7.99± 1.11 msec) positive notch (T) followed by much larger positive negative deflection (C) beginning at 11.61± 1.09 msec. Asphyxia or barbiturate injection selectively abolished C, but left T unaltered. It is suspected that T reflects the thalamocortical afferent activity (comparable to S of Perl and Whitlock), while C results from postsynaptic cortical discharge. Simultaneous with surface recording, unitary evoked spikes were recorded from 140 cells; 45 followed antidromic pyramidal stimulation at 100-200/sec, and were identified as "Betz" cells. Only one unit ("non-Betz") fired during T, and spike form suggested fiber origin; 28.1% of units began firing before peak positivity of C, 67.2% during interval from peak positivity to peak negativity, 14.7% later. Distribution of cortical delays of initial firing (latency spike minus latency T) did not differ significantly for "Betz" and "non-Betz" units; (minimum 2.5—2.8 msec). Because many units (particularly Betz cells) fired repetitively, probabilities of spiking irrespective of position of spike in train were computed for individual msec intervals. Summation of probabilities for population gave measure of probable number of spikes in each interval; 21.9% spiking occurred before peak positivity of C, 55.5% from peak positivity to peak negativity; 22.6% after peak negativity. Mean initial latency (msec) varied with depth and cytoarchitectonic layer. Although differences are small, the trend suggests initial activity in layers III and IV synaptically spreading both deep and superficially. There is also unexpected early activity in layer VI. (Aided by a grant B395, PHS.)

430. VECTORELECTROMYOGRAPHIC STUDY OF INTERCOSTAL MUSCLES IN HUMAN RESPIRATION. John E. Pauly,* David S. Jones,* Allan Jarach* and J. E. P. Toman. Depts. of Anatomy, Physiology and Pharmacology, Chicago Med. School, and Dept. of Anatomy, Stritch School of Medicine, Loyola Univ., Chicago, Ill.

Electromyographic studies of man by means of surface electrodes have shown the intercostal muscles to be active during forced inspiration, forced expiration, coughing and various movements of the trunk. The controversial question is whether the internal and external intercostal muscles act together or oppositely in producing these actions. An electromyographic analysis of the individual muscles while these actions occur would be desirable; however the difficulty of identifying these thin muscle layers located between the ribs and the unpleasantness of needle electrodes has always been a problem to the investigator. Therefore in the present study a special assembly of 4 surface electrodes was oriented in the chosen intercostal space, and opposite electrode pairs led off to separate amplifiers feeding the x and y axes of a cathode ray oscilloscope. The resultant loop patterns are highly characteristic for individual motor units; furthermore the average orientation of large numbers of units in massive discharge gives clues to the orientation of the muscle fibers. External and internal intercostals may be distinguished from each other and from overlying muscles. Typically there is intercostal silence in eupnea. Both the internal and external intercostal muscles in the midclavicular, anterior and midaxillary lines are active during forced inspiration and expiration and during extreme maintained inspiration and expiration. Patterns of involvement may vary over the thoracic wall, between subjects and even in adjacent interspaces.

431. A SIMPLE TEST FOR ASSAY OF PULMONARY DIFFUSION IMPAIRMENT. Louis J. Pecora. Occupational Health Program, USPHS, Cincinnati, Ohio.

An easy, reliable and quick screening method for determining functional pulmonary disability, due to impaired diffusion through the alveolar tissue, is desirable for clinical and field use. An ear oximeter measures arterial O_2 saturation in the subject resting and working. An index of diffusion impairment was determined as follows: DI = decrease % O_2 sat. after exercise/total ft lb work done x 10^5. Fifteen normal subjects and 59 patients, most of whom had lung diseases, believed to interfere with diffusion, were tested. The values for the 15 normals did not exceed 20 (median = 2). Sixteen patients recovering from pneumonia, miliary tuberculosis and uncomplicated bronchiectasis, not expected to show impairment, had

values in the normal range. Of 43 patients diagnosed as having pneumoconiosis, tuberculosis with fibrocalcification and emphysema, 30 had values ranging from 24-1112 (median = 126). Even though they were under treatment, 70% of the latter group gave values outside the range observed in normal subjects. It is recognized that this test is an indirect estimation of diffusion capacity provided also that no circulatory impairment exists.

432. SYMPATHETIC CARDIOACCELERATOR CENTER IN THE MEDULLA. Clarence N. Peiss. Dept. of Physiology, Stritch School of Medicine, Chicago, Ill.

Conventional statements of the nervous control of heart rate describe functionally related medullary centers which act via peripheral autonomic extensions, and by interaction with each other. It is commonly stated that the cardioaccelerator center lies in close apposition to the vasoconstrictor center on and just beneath the floor of the 4th ventricle. Electrical stimulation of the dorsal portion of the medulla in 30 cats with bilateral vagotomy has been carried out using stereotaxic procedures. Over 600 stimulations in this area have failed to elicit a primary cardioacceleration. In some animals, acute bilateral adrenalectomy and spinal cord transection has been carried out to eliminate secondary acceleration due to adrenal secretion. When the ventrolateral portion of the medulla and pons is similarly stimulated in the same animal, significant increases in heart rate are obtained, of a magnitude similar to that seen when the stellate ganglion is stimulated. These experiments raise serious doubt as to the existence of a medullary cardioaccelerator center.

433. FACTORS IN OXYGEN HIGH PRESSURE PULMONARY DAMAGE. Kenneth E. Penrod. Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.

The etiology of the pulmonary damage associated with exposure of small animals to high pressures of oxygen (OHP) is little understood. Albeti ubiquitous in long exposures at 3-6 atmospheres pressure, the origin of the atelectasis, edema, congestion and, in some cases, frank hemorrhage, is as yet obscure. The theory has been advanced that mucoidal secretion produces plugging of the distal airways with a resultant absorption of trapped gases peripheral to the obstruction bringing about focal atelectasis. An attempt has been made both to discover the stimulus to pulmonary secretion and to alter its production in OHP. A CNS component may be operative since CO_2 greatly increases the extent of the damage, but in guinea pigs and rabbits tying off the carotid arteries prior to exposure had no beneficial effect. The effect of inactivating the hemoglobin and the intermittent exposure to various gases and mixtures of gases as well as the response to mechanical insufflation will be discussed. (Work done under grant by Office of Naval Research.)

434. RAPID EVALUATION OF BASIC FUNCTIONS OF LUNG IMMEDIATELY AFTER THORACOTOMY. John F. Perkins, Jr., James B. Huffer,* William E. Adams,* John R. Benfield* and Joan S. Byrne.* Depts. of Physiology and Surgery, Univ. of Chicago, Chicago, Ill.

The recording oximeter-oxygen analyzer set-up for evaluating distribution of gas to the lungs using a nitrogen washout curve, and ventilation blood-flow relations by means of the curve relating arterial O_2 saturation to alveolar O_2 tension ("saturation-tension" curve, J. Appl. Physiol. 8:455, 1956; Fed. Proc. 14:1955 and 15:1956) has been modified so as to permit studies on patients in the period immediately after thoracotomy. A wheeled cart houses oximeter amplifier, control box, recording Pauling type O_2 analyzer, Speedomax recorder which automatically records O_2 saturations and O_2 tensions, gas meter and CO_2 absorber for O_2 consumption by the Margaria method. A separate cart carrying gas tanks, mixing valves and a 25-liter plastic Douglas bag with arrangement for continuous mixing is used to provide inspired gas mixtures containing 21-100% O_2, O_2 concentrations being monitored by visual reading O_2 analyzer. A Rahn alveolar sampling valve is mounted on this cart. The 2 pieces of equipment are wheeled into the patient's room starting the first day after operation, except in acutely ill patients. Since pure O_2 or O_2 enriched mixtures are being breathed during the major part of the tests, the latter are not particularly uncomfortable. Data will be presented showing functional changes immediately following surgery and permanent effects of removal of portions of the lung.

435. RESPONSE OF CROSSED MOTONEURONS TO ACTIVITY IN AFFERENTS FROM MUSCLE STRETCH RECEPTORS. Edward R. Perl. Dept. of Physiology, State Univ. of New York College of Medicine, Syracuse.

In spinal cats, activity in afferents from stretch receptors of a muscle acting on the knee altered the excitability of motoneurons innervating the corresponding muscle of the contralateral limb. The prominent effect produced by a single volley of impulses in the group I fibers of the muscle nerve was facilitation of the crossed motoneurons. The facilitation began 0.5-3 msec after arrival of the volley at the spinal cord and lasted about 10 msec. Ordinarily, this facilitation was preceded by inhibition. When the reflex path from the muscle nerve of a knee flexor to crossed knee flexor motoneurons was examined, inclusion of group II fibers in the volley from the nerve frequently added inhibition to the pattern obtained by group I activity alone. A similar inhibitory effect by group II fibers could not be demonstrated for the pathway from knee extensor to crossed knee extensor. Stretch of a knee flexor muscle with intact afferent innervation was also demonstrated to change the responsiveness of motoneurons of the contralateral knee flexors. Light static loads (25-65 gm) usually inhibited the crossed motoneurons, while heavier static loads and contraction of the muscle facilitated them.

436. INSTANTANEOUS DIMENSION-PULSE AND MEAN DIMENSIONAL CHANGES IN THE DOG KIDNEY. Sheridan L. C. Perry, Edward Hawthorne, William Kadetz and John P. Marbarger (intr. by Joseph L. Johnson). Dept. of Physiology, Howard Univ., Washington, D. C., and Aeromed. and Physical Environment Lab., Univ. of Illinois, Chicago.

It appeared that dimensional changes of the major diameters of the kidney are functions of changes in renal volume. A method has been devised for measuring instantaneous changes in length through various diameters of the kidney. This method utilizes a caliper-like strain gauge transducer as the sensing device. This transducer (may be applied across the desired diameter) is incorporated into an A-C bridge circuit. The output of the bridge is amplified, rectified and fed to one channel of a Sanborn Twin-Viso Cardiette. The same signal is fed through a Grass P4A preamplifier to the other channel. The caliper width is recorded through 2 channels; one having

response down to 0 cycles/sec (cps) and one having a response down 1/3 cps. In this way the instantaneous dimensional changes of the kidney can be recorded separately and simultaneously with very low and very high gains. The time constant of the high-gain recording circuit is long with respect to heart rate, but short with respect to rate of mean change of the renal dimensions. In vivo recordings of the variation of polar lengths of normal dog kidneys have been made using this system. Results indicate that the long polar dimension of the kidney decreases while the shorter anterio-posterior diameter increases with increase in volume. These changes are believed to be a result of a deformation of the surface of the kidney from a more ellipsoidal to a more spherical shape under the influence of increased intrarenal pressure and volume.

437. EFFECTS OF HIGH PROTEIN - HIGH VITAMIN SUPPLEMENTS ON CHOLESTEROLEMIA AND ATHEROGENESIS IN CHOLESTEROL-FED COCKERELS. R. Pick, J. Stamler and L. N. Katz. Cardiovascular Dept., Medical Research Inst., Michael Reese Hosp., Chicago, Ill.

This group of experiments was undertaken to test further the hypothesis that the balance in the diet between cholesterol + fat and proteins + vitamins may influence cholesterolemia and atherogenesis. The effects were analyzed of increasing the amounts of these essential nutrients (vitamins and proteins) in the diets of chicks fed starter mash plus a fixed content of cholesterol and cottonseed oil (1% and 5% respectively). Mash was supplemented with 7% each of the following defatted protein-rich materials: casein, soy protein, fish meal, liver meal. Brewer's yeast (7%) was also added, plus a complete supplement of individual vitamins, water- and fat-soluble, at 5 times the concentration usually employed in "purified" chick diets. Compared with birds ingesting cholesterol-oil mash without any protein-vitamin supplement, the cockerels on the enriched diet exhibited definite inhibition of hypercholesterolemia and atherogenesis. It is concluded that a high vitamin - high protein diet tends to protect chicks against the hypercholesterolemic and atherogenic effects of cholesterol-fat ingestion.

438. COMPARATIVE SPEED FORMATION, CARDIOVASCULAR AND MOTOR CONDITIONING. Teresa Pinto,* J. E. Newton,* and W. Horsley Gantt. Univ. of Chile, Santiago, and Johns Hopkins Univ., Baltimore, Md.

We have established cardiac conditional reflexes to pain, food and sexual stimulation which are specific quantitatively to intensity of stimulation. Cardiac Crs are more stable, lasting sometimes years after the motor or salivary have been lost. To answer whether the cardiac forms simultaneously with motor we studied 13 dogs. Motor Cr was recorded as any movement interpreted as referable to signal (the orienting reflex having been extinguished) whether specific or general. Results: cardiac and respiratory anteceded the motor and once formed were more persistent without reenforcement. The number of reenforcements for cardiac conditioning were, 3, 3, 3, 5, 4, 4, 4, 4, 4, 1, 1; for motor conditioning, 65, 90, 20, 9, 6, 10, 15, 15, 15, 15, 35, 4; average cardiac, 3.6; motor, 21. Included were 2 dogs deprived of both gyri cinguli and hemidecorticated. One phlegmatic dog formed no cardiac Crs. In spite of greater sensitivity of cardiac recording to motor, we feel warranted in concluding: the cardiac system conditions quicker to life experiences than does the somatic musculature; that emotional-autonomic learning is both more rapid to appear and slower to disappear than the specific muscular, an added evidence of the difference in the two kinds of adaptation, often leading to functional pathology, which we have characterized as schizokinesis.

439. SOME PROPERTIES OF FAT-FREE BODY OF THE GUINEA PIG. Grover C. Pitts. Dept. of Physiology, Univ. of Virginia School of Medicine, Charlottesville.

The carcasses of 47 adult guinea pigs were studied by dissection, freeze-drying and fat-extraction. Conclusions are based upon calculations made on 4 other published studies as well as our own. A correlation between fatness and fat-free body weight (FFBW) is shown. Specific gravity of and percentage in the FFB are calculated and their correlations with fatness and FFBW are tested. Also, data on the composition of the FFB with respect to particular gross components are presented.

440. RENAL OXYGEN CONSUMPTION IN ACIDOSIS AND ALKALOSIS. J. William Poppell* and Kathleen E. Roberts. Physiology Dept., Memorial Ctr. for Cancer and Allied Diseases, New York City.

Normally the renal A-V oxygen difference is fairly constant despite wide variations in renal blood flow. It is not known, however, whether changes in extracellular or intracellular pH would affect renal A-V oxygen difference. A series of experiments has been carried out on dogs to study the renal differences in A-V oxygen, pH and PCO_2 in the following experimental conditions: 1) acidosis, 2) alkalosis, 3) administration of Triiodothyronine, 4) carbonic anhydrase inhibition (Diamox), 5) phosphate loading and 6) KCL administration. The results of these studies are as follows: renal A-V oxygen difference was not significantly altered by KCL, phosphate, T-3, respiratory or metabolic alkalosis, respiratory or metabolic acidosis. Carbonic anhydrase inhibition by Diamox decreased the renal A-V oxygen difference in 9 out of 10 determinations. However, the decrease following Diamox was within one standard deviation of the control values and is of doubtful significance. Twenty-eight out of 45 determinations showed renal venous pH to be equal to or greater than that of the arterial blood. In the remainder the difference was no more than 0.02 units. In 60% of determinations, renal venous PCO_2 was within 2 mm Hg of arterial PCO_2. There was no correlation between arterial - venous pH or PCO_2 difference and renal oxygen consumption at any given level of renal blood flow.

441. OCCURRENCE OF GASTROINTESTINAL LESIONS IN BEHAVIORALLY CONDITIONED AND INTRACEREBRAL SELF-STIMULATED MONKEYS. R. W. Porter, J. V. Brady, D. G. Conrad and J. W. Mason (intr. by D. McK. Rioch). Div. of Neuropsychiatry, Walter Reed Army Inst. of Research, Washington, D. C.

Of 15 monkeys restrained in chairs and subjected to prolonged behavior conditioning and/or intracerebral self-stimulation experiments 9 have died from gastrointestinal lesions. The behavior conditioning studies were designed to elicit either the "anxiety" type emotional response or the avoidance of noxious electrical shocks. Intracerebral self-stimulation of various limbic system structures was carried out through implanted bipolar electrodes. While the program for each animal varied considerably, all were subjected to intensive experimental study for 2-8 wk.

The gastrointestinal lesions identified included 6 instances of duodenal ulceration, 3 of non-specific ulcerative colitis, 2 of gastromalacia and 1 of intestinal intussusception. There was no obvious relation between the type of experimental program employed and the gastrointestinal lesion induced. Five control monkeys subjected only to restraint for similar periods of time revealed no gastrointestinal disorders.

442. SUBSTITUTION OF LITHIUM FOR POTASSIUM IN ACTIVE CATION TRANSPORT ACROSS THE HUMAN ERYTHROCYTE MEMBRANE. Robert L. Post, Dept. of Physiology, Vanderbilt Med. School, Nashville, Tenn.

Evidence has accumulated that human erythrocytes actively transport 2 atoms of potassium inward per 3 atoms of sodium transported outward in a tightly linked system. Inward transport has been shown to accept ammonium, rubidium and, to some extent, cesium, in place of potassium. Outward transport has not been shown to accept any other element than sodium. This paper will present evidence that lithium is actively transported inward, although slowly. Erythrocytes were depleted of potassium by cold storage in a sodium medium fortified with glucose and nucleoside for 2 wk. They were then incubated at 37° in various media and net transport was determined by flame photometric analysis of the cells at intervals up to 6 hr. Cells were prepared containing mostly sodium and a moderate amount of lithium; when they were incubated in a medium of similar cation composition but lacking potassium, lithium moved into the cells and sodium out of the cells against concentration gradients. In control cells treated with strophanthin, which stops active transport specifically, these movements did not occur. Replacing external sodium with potassium depressed lithium entrance. The depression of active sodium transport induced by lack of external potassium was partly relieved by external lithium. (Supported by grant H-1974(c) from the Natl. Heart Inst., PHS.)

443. OXIDATION OF UNSATURATED FATS AS SOURCE OF ELECTRIC POWER OF CARDIAC PACEMAKER. George E. Price* and R. H. Beutner. Med. Research Lab., Des Moines Still College, Des Moines, Iowa.

Nerves or muscles not only transmit, but also generate impulses, e.g. in the cardiac pacemaker. The electric power supply for generation of impulses in a pacemaker is not directly produced by phospholipid splitting (which generates the action potential transmembrane metabolism). This power can be shown to be produced by oxidation of water-immiscible substances, such as unsaturated fats. As shown by Osterhout's experiments, a diminutive battery is formed, if an oxidizable lipoid of a membrane borders on a non-oxidizable lipoid of the same membrane, both lipoids being in contact with one saline solution. The nonoxidizable lipoid has a permanently negative p. d. where it borders on the saline; the oxidizable lipoid has an alternatingly negative or positive p. d. Rhythmic impulses are thus generated. As nutritional experiments show, deficiency of unsaturated fats leads to cardiac disease by producing coronary thrombosis, atheromata or other arterial pathology. But besides, congestive failure may appear in the absence of thrombosis or arterial disease. This may likewise be due to a deficiency of unsaturated or oxidizable fats, since the power supply of the pacemaker is inhibited by this deficiency. The heart will be arrested even without coronary thrombosis. We cannot substitute water-soluble oxidizable substances, as glucose or aminoacids for these fats. Water-soluble substances can only produce heat, when oxidized, but not the electric energy needed by the pacemaker. Predominant nutrition with saturated or hydrogenated fats depletes the store of unsaturated fats in the body, and will finally arrest the pacemaker.

444. MEAN DIFFERENCE IN OXYGEN PRESSURE BETWEEN MATERIAL AND FETAL BLOOD IN GOATS. Harry Prystowsky, Donald Barron, James Metcalfe and William E. Huckabee (intr. by Edgar J. Boell). Johns Hopkins, Baltimore, Md., Yale Univ., New Haven, Conn., Harvard and Boston Univ., Boston, Mass.

The relationship between maternal and fetal oxygen dissociation curves represents one factor in the oxygen transfer across the placenta. Such dissociation curves were constructed at various stages of pregnancy. Of 21 curves prepared with blood from 9 pregnant individuals all fell within the range of normal goat goats. The fetal curve characteristic of 65-136 days gestation was found to rise rapidly to a saturation of 70% and at that level it deviated sharply to the right, to flatten out and approach the horizontal at a saturation of 90%. These curves are quite to the left of the area characteristic of adult goats. Points on the curve prepared with blood from the fetus 139 days postinsemination fall to the right of the area characteristic of blood from fetuses 65-136 days, but some distance to the left of the area enclosing curve of the bloods of nonpregnant goats. The tendency of the curve to shift from the early fetal toward the adult type appears to manifest itself first at 139 days. Using these curves the oxygen pressures in the bloods of the umbilical artery, umbilical vein, uterine artery and uterine vein were estimated from the percentage saturation of the individual samples. With the values obtained the mean difference in oxygen pressure between the fetal and maternal placental bloods as estimated by a graphic method was 38±2 mm Hg.

445. FACTORS AFFECTING ACTIVITY CYCLES OF APICAL DENDRITES (CAT). Dominick P. Purpura, Edgar M. Housepian* and Harry Grundfest. Depts. of Neurological Surgery and Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.

The dendritic potential evoked by surface stimulation is the net result of opposing types of synaptic electrogenesis (Purpura and Grundfest J. Neurophysiol. 19:573, 1956). Variations induced by conditioning-testing or by low frequency surface stimulation are similarly related to different degrees of excitatory and inhibitory interaction under different experimental conditions and in different neocortical regions. Activity cycles of dendrites are characterized in unanesthetized, succinylcholine-paralyzed or encephale isolé preparations by brief inhibitory action (<30 msec.), followed by prolonged excitatory effects (80-120 msec.) which vary in intensity in different cortical regions. As a consequence of these differences, low-frequency (15/sec) stimulation of rostral cortical areas (anterior, posterior sigmoid) rapidly recruits early and late dendritic potentials. This is not encountered in intermediate or caudal regions (anterior, middle suprasylvian). Prolonged excitatory effects revealed in this manner are markedly altered by sub-anesthetic doses of Nembutal supplemented with $MgSO_4$, but are not significantly changed by volatile anesthetics. Whereas the conditioning response is augmented by the Nembutal, inhibition of the testing response is prolonged, lasting 100-120 msec. Inhibitory effects following surface stimulation

are also prolonged on eliminating sub-cortical or intracortical influences. These effects likewise differ in different cortical regions. The results indicate that barbiturates and cortical isolation precedures produce effects on overall dendritic activity determined by differences in intrinsic synaptic organizations.

446. PROBLEMS ASSOCIATED WITH A MAGNETIC TAPE RECORDING SYSTEM. Edward P. Radford, Jr., Paul E. Smith, Jr.* and Lucien Brouha. Physiology and Physics Sections, Haskell Lab. for Toxicology and Industrial Medicine, Newark, Del.

Simultaneous recording of several variables is becoming increasingly necessary in many fields of physiological research. At the same time more elaborate calculations from the recorded data are also needed. To speed up the processing of recorded data, we have obtained a commercial magnetic tape recording system using 1-in. tape on which 10 tracks may be recorded. Tape speeds of 0.75, 1.5, 7.5 and 15 in/sec are available for recording or playback. One channel is for audio recording. The data recording system is frequency modulated, and on one channel a reference frequency is recorded; the data tracks are compared on read-out to the reference frequency, thus minimizing errors arising from variations in tape composition and speed. The recorded data may be monitored and checked immediately. A stabilized high-speed analogue computing system is used for calculations. The accuracy of this method of recording and calculation depends primarily on good zero stability, freedom from noise and stable and linear calibration of the transducers, as well as stability of the computing units. The recorded data may not meet all these requirements and modification of the recording or computing techniques may be necessary. One method of dealing with nonlinearity of calibration (e.g. from a CO_2 meter) is to "linearize" during computation by using a function generator. Problems of instability of the zero level and calibration, noise and nonlinearity were all present with standard pneumotachograph recordings and studies of the pneumotachograph were carried out in an attempt to eliminate potential errors at the source.

447. THYROXINE INSENSITIVITY FOLLOWING HYPOTHALAMIC LESIONS IN RATS. Estelle R. Ramey and Maurice S. Goldstein. Dept. of Physiology, Georgetown Univ. School of Medicine, Washington, D. C. and Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

It has been shown that autonomic blocking agents such as dibenzyline may diminish the increase in oxygen consumption observed after treatment with exogenous thyroxine. It appears that there is a neural component in the overall metabolic response to the thyroid hormones. Electrolytic lesions were placed in the ventromedian nuclei of the hypothalamus of male rats. For the most part, the lesions were placed 1 mm to the right of the midline and 0.5 mm superior to the sphenoid bone. Such lesions may produce hyperphagia and obesity. Normal and experimental animals were injected with 1.5 mg/kg Na 1-thyroxine subcutaneously. Fifty-four hours after this single injection of thyroxine and following a 24-hr fasting period, the oxygen consumption of all the rats was determined. The rise in oxygen consumption of the normal rats in response to the exogenous thyroxine was 38% as an average. The average rise in oxygen consumption in those rats with hypothalamic lesions was 12%. The tachycardic response to thyroxine was consistent in normal animals and variable in the experimental animals.

448. PROPERTIES OF TURTLE HEMOGLOBINS. J. Raul Ramirez and Herbert C. Dessauer (intr. by Thomas Hernandez). Dept. of Biochemistry, Louisiana State Univ. School of Medicine, New Orleans.

Paper-electrophoretic patterns of amphibian and reptilian hemoglobins tend to parallel phylogeny. Hemoglobins of turtles from families Chelydridae and Kinosternidae appear to have one hemoglobin; those from Emydidae, Trionychidae and Testudinidae a number of hemoglobins. Two hemoglobins of the turtle, Pseudemys scripta elegans, were fractionated on a hanging curtain electrophoresis unit (barbital or borate buffers, pH 8.6, ionic strength 0.05, 15% glycerol). Seventy percent of total hemoglobin is found in the fraction that migrates slowly. Aliquots of each fraction and a laboratory mixture of both were subjected to analytical electrophoresis in barbital buffer. One fraction, Slow Hb, migrates at the rate of human hemoglobin S; the other fraction, Fast Hb, migrates at twice this rate. When the mixture is exposed to electrophoresis, Slow Hb migrates faster than "pure" Slow Hb; Fast Hb migrates slower than "pure" Fast Hb. A trail of pigment marks the paper between slow and fast components. Such interaction explains the trailing observed on electrophoretic patterns of freshly hemolyzed turtle blood. Both Slow and Fast Hb's combine with oxygen and contain no methomoglobin. Within limits of resolution of Beckman DU spectrophotometer, absorption spectra of oxy and reduced Slow and Fast Hb's are identical with those of human hemoglobin A. Specific extinctions based upon iron content are within 5% of those of hemoglobin A. In buffers of pH 12 Fast Hb denatures almost as rapidly as hemoglobin A, whereas Slow Hb is very alkali stable.

449. EFFECT OF ANEMIA ON PULMONARY DIFFUSING CAPACITY. J. Rankin,* R. S. McNeill* and R. E. Forster. Dept. of Physiology and Pharmacology, Univ. of Pennsylvania Grad. School of Medicine, Philadelphia.

Previous work has indicated that a) the overall rate at which the hemoglobin in the pulmonary capillary bed at any instant can form COHb/mm Hg of plasma CO tension, is of comparable importance to b) the rate of diffusion of CO across the pulmonary membrane per mm Hg of CO tension difference, in determining the diffusing capacity of the lung (D_L). Since a is proportional to the amount of hemoglobin present in the capillary bed, D_L should decrease in anemia, provided the rate (in terms of saturation) at which the individual red cell can form COHb/mm Hg plasma CO tension has not altered, and provided the decrease in blood hemoglobin concentration is not compensated by an equivalent increase in pulmonary capillary blood volume. We measured C_L by a modification of the Krogh 10-sec single breath CO technique at several alveolar O_2 tensions between 120 and 600 mm Hg in 5 patients with uncomplicated iron deficiency anemia and normal pulmonary function. Average D_L in the presence of anemia was decreased about 45% below the expected normal at all alveolar O_2 tensions. This decrease was mainly caused by a 60% reduction in the overall rate at which the intracellular hemoglobin in the capillary bed could form COHb/mm Hg CO tension in the plasma and not by any consistent change in the diffusing capacity of the pulmonary membrane. As the blood hemoglobin concentration increased with therapy, these values approached normal.

450. IMPORTANCE OF LIVER IN HYPERPHOSPHOLIPIDEMIA IN COCKERELS. R. E. Ranney and Sally E. Weiss.* Div. of Biological Research,

G. D. Searle and Co., Chicago, Ill.

Although SC-6924 (3-methoxy-16-methyl-1,3,5(10)-estratriene-16β, 17β-diol) significantly alters plasma lipid patterns in the chick, it has but slight estrogenic activity. Therefore further study of the effects of this compound on the phospholipid metabolism of the bird seemed desirable. Four-week-old cockerels were divided into 4 groups: I, normal birds treated with oil vehicle alone; II, normal birds treated with SC-6924 in oil; III, birds prepared for functional hepatectomy and treated with oil alone; and IV, birds prepared for functional hepatectomy and treated with SC-6924. After 30 hr, when the blood phospholipids of the fowl given SC-6924 had risen to a new steady state, groups III and IV were instantaneously hepatectomized and all animals were treated with inorganic P^{32}. At intervals of 2, 4 or 6 hr thereafter, animals of all groups were sacrificed and the specific activities of the phospholipids of several tissues were determined. Unlike the adult bird, the chick exhibited almost negligible contributions to plasma phospholipids from the extrahepatic tissues and treatment with SC-6924 did not modify this relationship. Similarly, renal phospholipid synthesis was unaffected by the hormone. The shape of the specific activity-time curve for aorta differed from that of the other tissues, showing a prolonged time lag after isotope administration. An apparent stimulation of phospholipid synthesis in aorta in group II indicated the possibility that the liver responded to SC-6924 by releasing to the circulation additional amounts of a precursor or cofactor important to this synthetic mechanism.

451. MICROCIRCULATORY EFFECTS OF TILTING AND ACCELERATION (MOTION PICTURE). Rita M. Rapp and Paul L. Yudkofsky (intr. by Brenton R. Lurz). Aero Med. Lab., Wright Air Develop. Ctr., Wright-Patterson AFB, Ohio.

Cardiovascular responses to gravitational or postural stress have been extensively studied. However, there are few direct observations on the behavior of the smaller blood vessels during such stress. To make such observations, the transparent cheek pouch preparation of the golden hamster (Mesocricetus auratus) offers an easily accessible and physiologically intact vascular bed. Cinephotomicroscopy has been used to study the arterioles, venules and capillaries during positive (head up) and negative (head down) tilting, as well as under various types of acceleration on the USAF human centrifuge. Motion picture sequences will be shown of the changes which occur in small blood vessel diameter and in blood flow under these various types of g-forces.

452. SHORT CHAIN FATTY ACIDS AS INHIBITORS OF HEPATIC METABOLISM. David Rapport, E. J. Masoro, J. M. Felts* and Sylvia S. Panagos.* Physiology Dept., Tufts Univ. School of Medicine, Boston, Mass.

Previous reports from this laboratory noted that butyrate and propionate severely inhibited the ability of rat liver slices to convert acetate-1-C^{14} to $C^{14}O$ Propionate caused less inhibition on the incorporation of acetate carbon into fatty acids than into CO_2, while butyrate had no inhibitory action on lipogenesis from acetate. In an attempt to characterize the nature of the inhibitory actions of these fatty acids, their effects on carbohydrate metabolism were studied. Butyrate and propionate caused a reduction in $C^{14}O_2$ production from evenly labeled glucose, glucose-1-C^{14} and glucose-6-C^{14}. Butyrate, in contrast to its effect on acetate metabolism, and propionate were both effective in inhibiting lipogenesis from glucose carbon. Unlike the action of these acids on acetate metabolism, the inhibition of lipogenesis was more severe than the reduction of glucose oxidation. Lactate-1-C^{14} oxidation was not affected by butyrate but was reduced by propionate. The conversion of lactate-2-C^{14} to $C^{14}O_2$ was somewhat reduced by propionate and butyrate but again lipogenesis from this compound was inhibited more than oxidation. These striking differences between the inhibitory actions of butyrate and propionate on hepatic acetate metabolism on the one hand and hepatic carbohydrate metabolism on the other seem to suggest that the metabolic path of the C2-fragment derived from these two sources must differ.

453. ANATOMICAL IDENTIFICATION OF SOMATIC SENSORY CORTICAL NEURONES RESPONDING WITH SHORT LATENCIES TO SPECIFIC AFFERENT VOLLEYS. M. Rayport (intr. by H. H. Jasper). Montreal Neurological Institute, Montreal, Canada.

The specific afferent evoked potential which is recorded from the cortical surface as a positive-negative deflection appears as a negative-positive wave when recorded from infra-granular regions of the cortex by a penetrating electrode. The surface-positive and deep-negative waves are considered manifestations of excitation occurring at the inward end of longitudinal conductors radially arranged with respect to the cortical surface. The anatomical identity of these neural elements is not known except by inference. An adaptation of the Prussian blue method of Hess and of Marshall has been devised to mark the intracortical location of the tip of glass pore microelectrodes used in recording from individual cortical neurones. Glass micropipettes (internal tip diameter 0.5 μ or less) are filled with $FeNH_4SO_4$, 3N solution. The resistance of such microelectrodes ranges from 9-20 negohms. No adverse physiological effects are apparent from the use of this electrolyte: extracellular records from a single neurone have been made during periods upwards of 2-1/2 hr, while intracellular records have been possible for over 5 min. The intracortical mark is made by passing a direct current of 0.05 μA for 45-65 min, with the microelectrode as the anode. Subsequently, the brain is perfused and fixed with K_4FeCN_6 formalin for not more than 72 hr. Paraffin serial sections are stained with Kernechtrot, a dye which gives histological results comparable to Nissl's stain except that the hue is red. Cortical neurones responding with latencies of 2.5-3.5 msec to specific afferent thalamic volleys were selected for staining and have been identified as medium or large pyramidal cells located just beneath the internal granular layer of the cortex.

454. PRESSURE FLOW RELATIONSHIPS IN TOTALLY PERFUSED DOG. Raymond C. Read, Hiroshi Kuida and John A. Johnson (intr. by W. E. Petersen). Depts. of Physiology and Surgery, Univ. of Minnesota Med. School, Minneapolis.

An extracorporeal pump-oxygenator system was used to determine pressure-flow relationships in the whole dog. Varying perfusion rates were obtained by diverting different fractions of the total flow into a measuring reservoir. Each rate was maintained for approximately 60 sec following which the animal was returned to the control flow to allow stabilization. Mean aortic and inferior vena caval pressures were continuously measured, the latter being held essentially constant. The effect of induced changes in peripheral resistance was determined in the same animals by repeating the pressure flow studies after pithing and treatment with hexamethonium, arfonad and aramine. A total of 20 pressure flow curves were

obtained in 8 dogs over a range of 0-160 cc-kg/min. Control curves were generally similar to each other being essentially linear over a considerable range but becoming convex to the pressure axis at high flows. Dogs with high vasomotor tone also showed a convexity to the pressure axis at low flows. This deviation from linearity was most evident in curves obtained after treatment with aramine. After pithing a linear pressure relationship was obtained throughout the entire range of flows, subsequent treatment with hexamethonium produced no change in the line. However, arfonad resulted in a decrement in the slipe of the pressure flow line. Despite the variation in vasomotor activity all pressure flow curves showed a positive pressure intercept, the magnitude of which appeared to be rated to the vasomotor tone.

455. EFFECT OF ESTROGENS ON RESPONSE OF CARDIAC GLYCOGEN TO DIGOXIN. W. O. Read. Dept. of Physiology and Pharmacology, Univ. of South Dakota, Vermillion.

Experiments with female rats indicate that digoxin causes a decrease of both trichloracetic acid (TCA) soluble and TCA-insoluble fractions of cardiac glycogen if administered following the onset of secual maturity. This effect is not evident in sexually immature rats or in ovarectomized adult females. Administration of estrogen to rats which have been ovarectomized before attainment of sexual maturity causes little effect on cardiac glycogen but when given in combination with digoxin causes an increase in all glycogen fractions. Estrogen treatment of rats which have been ovarectomized after attainment of sexual maturity causes a remarkable fall in all glycogen fractions whereas the combination of estrogen and digoxin treatment to such rats causes only a slight decrease in the TCA-soluble fraction. Digoxin administration to hypophysectomized female rats causes a decrease of all fractions of cardiac glycogen whereas estrogen alone causes an increase in the TCA-insoluble fraction with little change in the TCA-soluble fraction. Digoxin administered to combined ovarectomized-adrenalectomized rats causes an increase in the TCA-insoluble glycogen fraction and a slight fall in the TCA-soluble fraction. Estrogen alone to ovarectomized-adrenalectomized animals causes a decrease in the TCA-soluble fraction of cardiac glycogen but an increase in the TCA-insoluble fraction. Combined estrogen-digoxin treatment to ovarectomized-adrenalectomized rats causes a large rise in the TCA-soluble glycogen fraction with little or no change in the TCA-insoluble fraction.

456. EFFECTS OF CCl_4 ON ENZYME SYSTEMS OF RAT LIVER MITOCHONDRIA. Richard O. Recknagel and D. D. Anthony (intr. by F. Mautz). Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Rat liver mitochondria contain a 2,4-DNP activated ATPase which has an optimum at pH 7.5, requires no added divalent metal, is not inhibited by EDTA, and is destroyed in 1 hr at 37°C. After blendorization the above system is lost and is replaced by another ATPase which requires Mg ions, has an optimum at pH 8.5, and is stable at 37°C. When intact mitochondria are suspended at 0°C in 9.25 M sucrose saturated with CCl_4 (0.0063 M) the 2,4-DNP requiring system is completely lost and the Mg, ph 8.5 system is maximally activated. No ATPase activity is solubilized by CCl_4. The "ATPase transformation" was exactly paralleled by complete loss of α-ketoglutaric and glutamic oxidase activity when percentage saturation of CCl_4 or concentration of mitochondria was independently varied. "ATPase transformation" and increase in oxidative rate of intact mitochondria induced by 2,4-DNP were used as indices of mitochondrial damage in CCl_4 fatty liver. Extensive mitochondrial damage was evident at 20 hr following CCl_4 feeding, but not at 14 hr. Tota liver fat is doubled as early as 14 hr following CCl_4 feeding. These observations suggest that the mitochondrial alterations which were measured are not critical in the early stages of development of CCl_4 fatty liver. Observations of Christie and Judah (Proc. Roy. Soc. B 142: 241, 1954) that pyridine nucleotide dependent oxidations of mitochondria are depressed as early as 12-14 hr following CCl feeding could be confirmed, when assays were carried out at 37°C without added ATP. With added ATP at 30°C, the effect was not observed. (Supported by PHS Grant No. A-329-C4.)

457. INHIBITION OF d-AMINO ACID OXIDASE BY TUMOR EXTRACTS. D. V. N. Reddy (intr. by C. V. Winder). Dept. of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia Univ. and Francis Delafield Hosp., New York City.

The lack of d-amino acid oxidase activity in tumor tissues has been attributed to a deficiency of apo- and/or coenzyme of this system. (Shack. J. Natl. Cancer Inst. 13:829, 1943). However, it is possible that tumor tissue contains inhibitors of d-amino acid oxidase. Accordingly, an investigation was undertaken to test this hypothesis. Tumor extracts, which were prepared by centrifuging 10% homogenates in Krebs-Ringer phosphate buffer, pH 7.4, at 20,000 g for 12 min were tested with a standard preparation of hog kidney amino acid oxidase. All tumors tested inhibited the oxidase from 25-85%. To elucidate the mechanism of inhibition, extracts of adrenocarcinoma 755 were used; the inhibition in this case was 80%. Under similar conditions, extracts of normal rat liver and kidney failed to inhibit the oxidase. The inhibitor was inactive at pH 8.3 and was completely destroyed by heating for 4 min in a boiling water bath. When the extracts were centrifuged for an hour at 140,000 g, the major part of the inhibitory activity was found in the particulate fraction. Ultrafiltrates of tumor extracts were totally inactive. The inhibition of d-amino acid oxidase by tumor extracts was partially reversed by the addition of flavin adenine dinucleotide (FAD). On the basis of incubation studies followed by chromatographic separation, it has been shown that the inhibitor acts on the cofactor of the oxidase to yield the inactive flavin mononucleotide (FMN) and adenosine triphosphate (ATP). FMN is further split to riboflavin. It is suggested that the inhibitor is a pyrophosphatase.

458. ARTERIAL WALL ELECTROLYTES IN DESOXYCORTICOSTERONE HYPERTENSION. Paul D. Redleaf* and Louis Tobian. Dept. of Medicine, Univ. of Minnesota, Minneapolis.

An increase in sodium and potassium content of the aorta wall is associated with desoxycorticosterone hypertension in the rat. The present study was undertaken to determine the correlation of hypertension per se with the development of these chemical alterations. Two injections of desocycorticosterone trimethylacetate (DCTMA) were given to Wistar rats at monthly intervals. Twenty-one rats received supplementary sodium chloride; in 10 rats sodium intake was drastically curtailed. A control group received a low sodium intake without DCTMA. After obtaining blood pressures during the 7th experimental week, the aortas were obtained for analysis. An elevation of sodium occurred in the aortas of all rats

receiving DCTMA plus supplementary salt. This was significantly greater in severely hypertensive than in equivocally hypertensive rats ($p < .02$). Aorta potassium content was strikingly increased in severely hypertensive rats compared with either group on alow sodium intake ($p < .0004$). The aortas of the equivocally hypertensive group had a potassium content below control valves. Sodium restriction completely prevented the development of hypertension among DCTMA-treated rats, whose aorta electrolytes remained normal. The chemical alterations in the aorta of equivocally hypertensive rats are similar to those produced in skeletal muscle by dexocycorticosterone. The elevation of potassium in severely hypertensive rats appears directly related to hypertension per se since it did not occur in the equivocally hypertensive group.

459. EFFECT OF POSITION OF THE BODY ON ALVEOLAR GAS COMPOSITION. E. A. Reed. Physiology Dept., Hahnemann Med. College, Philadelphia, Pa.

Mongrel dogs were anesthetized with Nembutal (35 mg/kg, i.p.) and placed supine on a tilt board. A tracheal catheter with inflatable cuff was inserted to a level just above the bifurcation. A Douglass valve was fitted to the catheter and connection was made to a Peck-Waller Tidal Volume Recorder through which the dog breathed air. Kymograph records of tidal volumes were made. A polyethylene tube was inserted through an air-tight side arm to the tip of the catheter. The dog was tilted alternately head down and head up 30°. From 10-30 min after turning gas samples were taken. At the end of expiration gas was drawn into 10-ml bottles where it was stored over mercury under slight pressure. The dead space in the system was flushed with alveolar gas about 30 sec previously. Samples then were analysed in the 0.5 ml Scholander analyser from the % of CO_2 and of O_2. In 14 experiments on 7 dogs the following average data were found.

	Head Up	Head Down	% Diff.
Al. CO_2	6.96%	6.43%	-8
Al. O_2	11.70%	12.64%	8
Resp. freq.	16.3/min	25.5/min	56
Min vol.	2,376 ml	3,054 ml	28

460. ENZYME SYNTHESIS IN VITRO. John M. Reiner. Div. of Labs. and Research, New York State Health Dept., Saratoga Springs.

Cell membranes and cell membrane fragments have been prepared by mechanical and chemical treatment of cells of Escherichia coli. Such preparations, when supplied with appropriate sources of energy and raw materials, were able to synthesize the enzyme beta-galactosidase. The presence of the natural substrate, lactose or of a lactose analog or similar inducer is required for synthesis. A mixture of amino acids and some form of nucleic acid precursor (such as a mixture of purine bases) is also required. The kinetics of synthesis has been studied in relation to the accompanying changes in nucleic acid metabolism in the preparations. The effects of purified preparations of deoxyribonucleic acid and ribonucleic acid derived from lactose-grown and glucose-grown cells were investigated. The synthesis is inhibited by a number of compounds, including specific inhibitors of protein and nucleic acid metabolism.

461. TRANSCAPILLARY EXCHANGE OF POTASSIUM IN MAMMALIAN SKELETAL MUSCLE. E. M. Renkin. Lab. of Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

A method for studying blood-tissue exchange of potassium has been developed, based on the Fick principle and utilizing measurements of blood flow and arteriovenous difference of tracer K^{42}. This method has been used to measure K^+ exchange rates in isolated, blood-perfused dog gracilis or gastrocnemius muscles at rest, during and after activity. In the resting muscles, K^+ exchange is sensitive to changes in capillary blood flow. Thus K^+ exchange is increased by increasing arterial pressure, though to a lesser extent than is the blood flow and for a given arterial pressure, exchange is increased by vasodilatation and decreased by vasoconstruction. During and after periods of intermittent tetanic contraction, K^+ exchange is increased roughly in proportion to the rise in blood flow above the resting level. It is concluded that control of the capillary circulation by purely hemodynamic factors can be an effective means of adjustment of transcapillary exchange to meet the metabolic needs of the muscle.

462. NATURE OF METABOLITES OF EPINEPHRINE CONTAINING ISOTOPIC CARBON. Oscar Resnick* and Fred Elmadjian. Worcester Fndn., Shrewsbury and Worcester State Hosp., Worcester, Mass.

Schizophrenic and normal subjects were infused with β-C^{14}-dl-epinephrine-d-bitartrate (E) for periods of time ranging from 5-39 min. The cardiovascular responses may be correlated with the presence and absence of the infused E in the blood and thus indicate a very rapid removal of E from the circulation. The infusion of E resulted in an increased urinary excretion of biologically active material which occurred during the first few hours after the onset of the infusion and was found to represent only 1-3% of the infused E. During this same period of time, approximately one third of the infused E was receovered in the urine as radioactive metabolites. By 30 hr following the onset of the infusion, essentially all of the radioactivity infused could be accounted for in the urine. Thus, it may also be possible to correlate the prolonged excretion of E metabolites with the effects of E on certain metabolic processes which have been observed 24-28 hr after the administration of E. Attempts to resolve and characterize the radioactive metabolites of E appearing in the urine have been undertaken. Tentatively, one may assume that the metabolites of E have the alpha and beta carbons intact and attached to the benzene nucleus and the hydroxyls at the 3- and 4-positions either intact or conjugated. Finally, the solubility characteristics of the radioactive metabolites obtained from our patients are compatible with the possibility that some of these metabolites may be catechols. (Aided in part by a grant from the Army Med. Research and Development D., Contract no. DA-49-007-MD-438, Ford Fndn. and Natl. Assn. for Mental Health.)

463. INFLUENCE OF THERMAL ENVIRONMENTS ON RECOVERY FROM EXPOSURE TO SEVERE HEAT. M. L. Riedesel,* H. S. Belding and D. M. Ross.* Dept. of Occupational Health, Graduate School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

Semi-nude subjects walked slowly for 30 min in heat which resulted in production of 0.6 liters of sweat and progressive rises of rectal temperature to 100.6°F (average); skin temperature to 100°F; and heart rate to 140 beats/min. Recovery from these exposures was followed during 1 hr of rest under 4 different environmental conditions: a) dry bulb 96°F

and vapor pressure 25 mm/Hg; b) dry bulb 86°F and vapor pressure 25 mm/Hg; c) like b, except vapor pressure 12 mm/Hg; and d) dry bulb 76°F and vapor pressure 12 mm/Hg. Greater strain accompanied recovery under condition a) as evidenced by a greater sweat rate, more heart beats, slower fall in rectal temperature, lesser fall in skin temperature and greater sensation of warmth. Differences between the other recovery conditions were significant in only a few instances, but the trends consistently indicated progressively less strain for b, c and d, in that order. Recovery from similar exposures of the same 4 men while wearing cotton shirt and trousers was studied under recovery conditions b, c and d. Under b there were more heart beats during the recovery period and the men felt warmer, but by the other criteria the recovery patterns for b, c and d were similar. In the same environment the strain of recovery was not generally different whether the men were clothed or semi-nude.

464. EFFECTS OF SILVER IONS ON OXYGEN EQUILIBRIUM OF HEMOGLOBIN. Austen Riggs (intr. by John Emerson Davis). Dept. of Zoology, Univ. of Texas, Austin.

The hemes of vertebrate hemoglobins interact with one another in such a way that oxygenation of one heme facilitates the oxygenation of a second. Intact -SH groups appear to be essential for this interaction and the effects of various mercurials have been studied (Riggs. and Wolbach. J. Gen. Physiol. 39:585, 1956). These experiments indicate that all mercurials examined tend to reduce heme-heme interaction. However, the valence of the mercurial appears to determine whether the mercurial increases the overall oxygen affinity, which is defined here as the reciprocal of the oxygen pressure required to half-saturate the hemoglobin with oxygen. Thus mercuric chloride increases the oxygen affinity at a concentration of 2 moles/mole of hemoglobin, while p-chloromercuribenzoic acid (PCMB) has almost no effect on the overall oxygen affinity at similar concentrations. Silver ions, being univalent, might be expected to have effects similar to those of PCMB. Present experiments indicate, however, that the effects of silver ions more closely resemble those of mercuric chloride. Two factors make the determination of the oxygen equilibrium difficult in the presence of silver. First, low concentrations of silver alter the absorption spectrum of hemoglobin much more than do mercurials. Second, hemoglobin gradually loses its oxygen capacity in the presence of silver. Mercurials do not appear to reduce the oxygen-binding capacity of hemoglobin except in the presence of urea. The mechanism whereby silver reduces the oxygen capacity is being studied and will be discussed.

465. CARDIOVASCULAR HEMODYNAMICS OF CONSEQUENCE TO CROSSED CIRCULATION IN DOGS. W. J. Roberson* and S. M. Horvath. Dept. of Physiology and Cardiovascular Lab., State Univ. of Iowa, Iowa City.

This study was undertaken to assess the responses of the cardiovascular system of the dog to crossed circulations. Two A-V shunts were constructed with polyethylene tubing connecting the central end of the carotid artery of 1 animal to the peripheral end of the jugular vein of the second. Each pair of animals were of approximately identical weights. Control observations were made on each intact animal and then the shunts, permitting free exchange of blood between the paired animals, were opened for 1 hr. After which these shunts were closed returning the animals to their original circulatory pattern and studies were continued for an additional hour. Upon opening the shunts the volume of blood flow through the carotid artery was at least twice normal and remained above control levels throughout the period of free crossed circulation. A drop of flow was noted after the first 10 min followed by a gradual return toward the initial high values. When the shunts were closed, the carotid blood flow immediately dropped and persisted at below control levels. The arterial blood pressure in these animals showed no initial change from control, but some 10-15 min of free crossed circulation a marked fall to as low as 40 mm Hg occurred. These pressures gradually returned toward control levels during the remainder of the open-shunt period and failed to return to control levels during the follow-up period.

466. RELATIVE BIOLOGICAL EFFECTIVENESS OF HEAVY PARTICLES FROM THE $B^{10}(n,\alpha)Li^7$ REACTION FOR SKIN LESIONS IN SWINE. J. S. Robertson, V. P. Bond,* E. P. Cronkite, O. D. Easterday* and S. M. Lippincott.* Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

The severe epidermitis produced by heavy particles from the $B^{10}(n,\alpha)Li^7$ reaction in patients and in swine has been reported previously by the present authors., Swine were injected intravenously with 30 μg of B^{10}/gm of body weight and irradiated with 5 x 10^{12} neutrons/cm^2, giving a surface dose of 1400 rad from α and Li particles plus 560 rad from other neutron reactions and gamma rays and leading to severe skin damage in about 10 days. To determine the relative biological effectiveness (RBE) of the heavy particle irradiation, doses of 500 r to 9000 r of 250 kvp x-rays (1 mm Al filter, HVL 0.13 mm cu) were delivered to 23 areas of the skin of 3 swine. Doses upwards from 2000 r produced lesions having a gross appearance comparable to that of those observed following neutron capture irradiation. Microscopic examination of sections of skin taken 21 and 30 days after exposure to x-ray does not show an exact correlation of severity with increasing dosage. The lesions studied showed focal degeneration or more widespread complete destruction of the epidermis with varying degrees of destruction of the dermis. At 2000 r hyperkeratosis and parakeratosis are present. At 2500 r there is a focal epidermal necrosis and at 3000 r there is bullous formation and areas of complete epidermal degeneration with extension into the outer portion of the dermis. Grossly and microscopically the lesions produced by x-ray doses of 2500 to 4000 r are comparable with those observed from the neutron capture irradiations. The RBE for irradiation from the heavy particles from the $B^{10}(n,\alpha)Li^7$ reaction is thus about 1.5-2.0 for the production of skin lesions in swine. (Supported by the U.S. Atomic Energy Comm.)

467. ALVEOLAR GAS CONCENTRATIONS AND RESPIRATORY CENTER SENSITIVITY TO CO_2 DURING NATURAL SLEEP. Eugene D. Robin, R. D. Whaley, C. H. Crump and D. M. Travis (intr. by Chester E. Leese). Dept. of Medicine, Harvard Med. School and Med. Clinics, Peter Bent Brigham Hosp., Boston, Mass.

An infrared CO_2 analyzer has been utilized to study changes in alveolar gas concentrations and respiratory center sensitivity to CO_2 during natural sleep in normal subjects. Sleep is associated with an increase in $PACO_2$. There is a decrease in total ventilation, alveolar ventilation, CO_2 production and O_2 consumption during sleep. Despite a small decrease in PAO_2

the level is such that hypoxia does not occur. Respiratory quotient and respiratory rate are largely unaffected by sleep. Changes in the rhythm of respiration are not uncommon. Respiratory center sensitivity to CO_2 is markedly decreased during sleep. Observations of this decrease in the ventilatory response to CO_2 indicate that it occurs promptly after sleep is established. Despite a marked decrease in the ventilatory response to CO_2 no component of peripheral chemoreceptor control of breathing during sleep can be demonstrated. These data indicate the following: 1) the "hypoxic" theory of the genesis of sleep is untenable; 2) the "hypercapnic" theory of the genesis of sleep is untenable; 3) the mechanism of the hypercapnia of natural sleep is at least partially based on a markedly decreased respiratory center sensitivity during this state; 4) it is probable that this depression of sensitivity is related to a decrease in the number of afferent impulses entering the respiratory center. (Supported in part by the Natl. Heart Inst., PHS.)

468. INFLUENCE OF ASCORBIC ACID ON DISTRIBUTION OF C_{14}-LABELED CHOLESTEROL IN GUINEA PIGS. Robert J. Robinson, William J. West and Walter M. Booker (intr. by E. G. Weir). Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.

Distribution and turn-over studies which we have presented previously have demonstrated species variation among dogs, rats and guinea pigs. Also, we have very recently shown that scorbutic guinea pigs administered C_{14}-labeled cholesterol show a greater distribution of the substance at the adrenal than in normal guinea pigs. When scorbutic guinea pigs are administered ascorbic acid as the cholesterol is administered, the cholesterol distribution at the adrenal is similar to that of normal animals. In the present study we are following the distribution of cholesterol 24 hr after cold stress at the liver and at the adrenal gland in scorbutic animals, normal animals and scorbutic animals administered ascorbic acid just prior to stress. It appears that the normal guinea pigs still show much less cholesterol at the adrenal 24 hr after stress than in the scorbutic guinea pig. It also appears that when scorbutic animals are given ascorbic acid, the chooesterol at the adrenal follows the pattern of the normal animal. Results also show higher distribution of cholesterol at the liver in normal than in scorbutic guinea pigs. Efforts are being made to follow the precursor role of cholesterol for the adrenocortical hormone in the guinea pig and the possible role of ascorbic acid.

469. CHANGES IN O_2 REQUIREMENTS OF MEN DURING EXHAUSTING RUNS. S. Robinson, D. L. Robinson* and R. J. Mountjoy.* Dept. of Physiology, Indiana, Univ., Bloomington.

In 4 experiments a man ran on the treadmill at 13.3 mph for the exact times of 1, 2, 2.4 and 3 min respectively. In previous trial runs this speed was found to exhaust the runner in 3 min. For each run his O_2 intake and O_2 debt were determined by the open-circuit method and samples of venous blood were drawn before and 4 min after work and analyzed for lactic acid. Both the runner's O_2 requirement and his rate of accumulation of lactic acid were found to decrease in the second minute of a 2-min run at constant speed and then to increase markedly in the last half minute as he approached exhaustion. His O_2 requirement for the run was 7.8 liters in the 1st min, 6.9 in the 2nd minute, 7.5 l/min in the period 2-2.4 min and 14.1 l/min from 2.4-3 min. Corresponding elevations of blood lactic acid were 47,44,36 and 82 mg%/min for the respective periods of the run. These results are confirmed by experiments on a second subject. It is clear from the data that for best performance in a middle distance race the runner should follow a pace which will delay until near the end of the race the sudden change in physiological state in which the energy cost of running and the development of fatigue are so greatly accelerated.

470. ABSORPTION OF CR-51 TAGGED ERYTHROCYTES FROM THE PERITONEAL CAVITY. D. B. Rochlin, W. S. Blakemore and A. Cary (intr. by Jonathan E. Rhoads). Harrison Dept. of Surgical Research, Univ. of Pennsylvania School of Medicine, Philadelphia.

Erythrocytes and other small particles of the size of 2-20 micra are absorbed intact from the peritoneal cavity and later appear in the circulating blood. Intraperitoneal transfusion has been used in other clinical centers for the transfusion of infants and may be of value in the management of patients with intraperitoneal hemorrhage where unclotted blood is left in the peritoneal cavity at the time of operation. This study was undertaken to determine: 1) the rate of absorption of intraperitoneally injected red cells; 2) the survival times of these cells in the circulation; and 3) the route of absorption of these cells. Fifty, 100, 250 and 500 ml of an erythrocyte saline suspension were injected into the peritoneal cavity of mongrel dogs. In these studies 70±5% of the injected red cells were absorbed into the intravascular space with the maximum absorption occurring at time intervals up to 100 hr after injection. Studies are now in progress to determine the route of absorption of erythrocytes following injection into the peritoneal cavity. The survival times for these cells are equal to erythrocytes transfused intravenously.

471. DYNAMICS OF ATRIAL SEPTAL DEFECTS. S. Rodbard and R. Kohn.* Univ. of Buffalo Chronic Disease Research Inst., Buffalo, N. Y.

Opening of an atrial septal defect in a circulation model, in which a wide variety of cardiovascular conditions can be simulated (Am. Heart J. 51:325, 1956), usually resulted in a late systolic shunt to the right. This was due to the fact that the right ventricular stroke output quickly traversed the pulmonary vascular bed, entering the left atrium during late systole when right atrial filling was not equivalent in volume. An increase in the pulmonary vascular resistance or capacity reduced this effect and caused the shunt to continue into early diastole. Simple dilatation of a chamber, without mural thickening, permitted a greater diastolic inflow into it and thereby enhanced the tendency to a diastolic shunt in the direction of the dilated chamber. Thus, right ventricular dilatation was uniformly associated with a diastolic shunt to the right. By contrast, thickening (hypertrophy) of a ventricular wall reduced the shunt to that chamber. For example, a progressive increase in the number of layers of rubber comprising the right ventricle reduced the shunt to the right and then reversed it. When a ventricle failed to empty completely (failure) this tended to reduce the shunt in its direction. Tricuspid stenosis increased the shunt to the left during boty systole and diastole, while mitral stenosis produced a shunt to the right. The diastolic shunts were also affected by the relative impedances to inflow into the atria; a dilated atrium permitted a shunt in its direction, while thickening of an atrial wall shunted the flow to the opposite side. A shunt to the right was enhanced during systole by mitral insufficiency and during diastole by aortic insufficiency,

although either lesion produced a continuous shunt. Use of the model thus aids in clarifying some of the mechanical factors which determine shunting in septal defects.

472. EFFECT OF EXTRA-CELLULAR pH ON MUSCLE ELECTROLYTES. Terence A. Rogers* and Wallace O. Fenn. Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Pairs of excised muscles from both frogs and rats were incubated in nutrient media at various hydrogen ion concentrations and then the remaining sodium and potassium contents were compared. Rat diaphragm muscles which were incubated at a pH of 6.7-6.8 invariably lost more potassium than their controls at pH 7.4. The extra potassium loss was smaller and less consistent when the acidotic medium was at a pH of 7.1. The sodium concentration showed great variability, presumably due to the high concentration of interstitial sodium, but when 90% of the sodium chloride in the medium was replaced by sucrose, it was found that the acidotic muscles contained less sodium as well as less potassium. The same observations were made on frog sartorious and semi-tendinosus muscles. The potassium efflux from rat diaphragm in acidotic solutions could be checked by transferring the muscle to another flask containing medium at pH 7.4. In some experiments the medium was sampled at intervals and it was found that the potassium loss was actually reversed when the pH was changed to 7.4. The effect of acidosis on the intra-cellular potassium seemed to be the same whether it was due to a low bicarbonate concentration at constant pCO_2 ("metabolic acidosis"), or to an increased pCO_2 with a constant bicarbonate concentration ("respiratory acidosis"). These experiments support the hypothesis that a relative acidification of plasma is neutralized by an exit of both sodium and potassium ions from the tissue cells.

473. INFLUENCE OF PHARMACOLOGIC AGENTS ON CAPILLARY-AVA FLOW DISTRIBUTION IN THE PERFUSED RABBIT EAR. P. A. Rondell, L. E. Palmer and D. F. Bohr (intr. by P. A. Wright). Dept. of Physiology, Univ. of Michigan, Ann Arbor.

A microsphere technique (Am. J. Physiol. 179: 323, 1955) was used to evaluate possible changes in the distribution of flow between capillary and arteriovenous anastomoses (AVA) circuits in response to arterial infusion of several vaso-active agents. Serotonin (.5γ/min) and Isuprel (35γ/min) significantly ($P < .001$ in either case) reduced the relative AVA flow, indicating a preferential constrictor action on the musculature of these channels. Infusion of histamine (.5γ/min), Pitressin (.0024 U/min) or acetylcholine (6.5γ/min) produced no significant change in the relative AVA flow. The observations suggest that the smooth muscle of both the AVA and of the metarterioles distal to AVA are equally responsive to these drugs. With respect to the total flow through the terminal vascular bed, all agents except acetylcholine were markedly constrictor. (Supported by research grant PHS H-1481 C from the Natl. Heart Inst., PHS.)

474. PULMONARY VASCULAR RESISTANCE AS FUNCTION OF RIGHT VENTRICULAR, LEFT AURICULAR, INTRAPULMONARY AND PLEURAL PRESSURES. Albert Roos, L. J. Thomas, Jr.,* E. L. Nagel* and D. C. Prommas.* Lab. of Thoracic Physiology and Depts. of Surgery and Physiology, Washington Univ. School of Medicine, St. Louis, Mo.

Since Poiseuille initiated the study of the effect of lung inflation upon vascular resistance (R), the nonlinearity of the P.-F. relation and the importance of left auricular pressure (V.P.) have become known. No clarity exists about the relative effects on resistance of lowering pleural (P.P.) vs. elevating intrapulmonary (I.P.) pressure. We have made a quantitative examination in the single isolated dextran-perfused dog lung of the effect of V.P., P.P. and I.P. upon flow over the range of 0.5 to 3000 cc/min. Using an expanded vertical tube arrangement with automatic recording, the P.F. plots on changing each of the 3 parameters in succession were obtained. Stability was checked periodically and flow rates were within physiological limits. Some of the results are as follows: at the same increase in lung volume, R increases 15-20 times as much when distension is produced by elevating I.P. than by lowering P.P. At V.P. minus I.P. equals about 6 cm H_2O, R becomes independent of flow at any lung volume. At still higher V.P., the P.F. plot is concave to the pressure axis, and the modest enhancement of flow is now due to decreased resistance on the venous side only. Thus, resistance increase from raising I.P. was quantitatively divided into a stretch deformation and a compression deformation component of the vascular bed. The contributions to a change in P.F. of "arterial" and "venous" resistance were obtained.

475. DETERMINATION OF ADRENALINE AND NORADRENALINE IN AQUEOUS SOLUTION. Sidney Roston (intr. by Joseph Blair). Army Med. Research Lab., Fort Knox, Ky.

A method for the determination of adrenaline and noradrenaline in aqueous solution has been developed which depends upon differential oxidation of adrenochrome and noradrenochrome rather than upon differential formation of these substances. The determination of adrenaline is thus made more independent of noradrenaline and vice versa. The method involves preliminary oxidation of the catechol amines with potassium ferricyanide at pH 5.5. The transformation of noradrenochrome to noradrenolutine in 0.5 N NaOH is considerably slower than the almost instantaneous formation of adrenolutine from adrenochrome. Exposure of the noradrenochrome and adrenochrome to the same alkalinity for 30 sec followed by the addition of 2 mg of ascorbic acid per 10 ml of solution results in a 95% loss of the adrenolutine fluorescence and only 30% loss of the noradrenolutine fluorescence. As a result, one gamma of noradrenaline yields the same fluorescence as 10 gamma of adrenaline. On the other hand, addition of 32 mg of ascorbic acid simultaneously with the NaOH to the adrenochrome and noradrenochrome solutions results in the retention of 90% of the adrenoluteine fluorescence, but only 36% of the noradrenolutine fluorescence, with 1 gamma of adrenaline then being equivalent in fluorescence to 4.5 gamma of noradrenaline. Solution of 2 linear simultaneous equations yields values of the individual catechol amines in mixtures. Between .03 and 1 gamma of the catechol amines, the range determined, the standard deviation of the method is within 10%.

476. SIMULTANEOUS DETERMINATION OF BLOOD PRESSURE AND BLOOD pH IN SUSPECTED PHEOCHROMOCYTOMA. Grace M. Roth, Herbert A. Roedling* and Richard M. Shick.* Mayo Clinic and Mayo Fndn., Rochester, Minn.

This investigation was stimulated by the work of E. B. Brown, who confirmed earlier work of Burget

and Visscher showing that the increase in blood pressure in animals after administration of epinephrine was decreased when the blood pH was decreased. Because epinephrine and norepinephrine are the pressor amines released from pheochromocytomas, the question arose as to whether changes in blood pH might produce equivocal results in pharmacologic tests for such tumors, particularly after administration of antihypertensive drugs and sedatives. We did 430 determinations of blood pH with simultaneous measurement of blood pressure on 325 patients screened for pheochromocytoma. Of these patients, 114 (66 males and 48 females) had not received medication; the average blood pH in this group was 7.38±0.003 and 7.40±0.003, respectively. The patients who had received medication had an average blood pH of 7.39± 0.005. Multiple determinations of both blood pH and blood pressure were done on 100 of these patients. A consistent increase in blood pH with only a slight increase in blood pressure occurred after use of regitine alone and a combination of histamine and regitine; this occurred with or without preliminary medication. A group of 16 patients had an average blood pH of 7.29; this increased to 7.40 after oral administration of 40 gm of sodium bicarbonate. The blood pH has not had a definite effect on the increase in blood pressure in patients undergoing these tests, but circulating pressor amines apparently have not been present.

477. PULMONARY AND SYSTEMIC VASCULAR RESPONSES TO 5-HYDROXYTRYPTAMINE (SEROTONIN). A. M. Rudolph and M. H. Paul (intr. by W. T. Goodale). Dept. of Pediatrics, Harvard Med. School and Children's Med. Ctr., Boston, Mass.

Serotonin creatinine sulfate solutions were continuously infused at varying rates into the venous system of intact dogs. Pulmonary and systemic pressures and, in some instances, left atrial pressures were continuously recorded and cardiac output was repeatedly measured. Infusion rates of 20 μg/kg/min resulted in a two-fold increase of pulmonary arterial pressure. Maximal effects were noted with an infusion rate of 150 μg/kg/min. In 11 experiments there was a 300% increase in mean pulmonary pressure, a 28% decrease in mean systemic arterial pressure and a 60% rise in cardiac output. Calculated pulmonary resistances increased markedly to 500% of control levels. In contrast, systemic vascular resistance decreased by 55%. The pulmonary and systemic effects were maintained as long as the infusion was continued; 5-hydroxytryptamine is thus a powerful vasoconstrictor of the pulmonary vessels. When the drug is administered by continuous infusion, the initial effects on systemic arterial pressure are variable, but after 2-3 min it always produces a reduction in mean systemic arterial pressure and a fall in peripheral resistance.

478. GLUCOSE OXIDATION IN PROSTATIC TISSUE FROM NORMAL AND HYPOGLYCEMIC DOGS AND THE EFFECT OF LSD. Guilford G. Rudolph* and Norman S. Olsen. Radioisotope Service and Research Lab., VA Hosp., Nashville, Tenn.

In vitro metabolism of glucose by dog prostate slices has been studied with glucose-1-C^{14} and -6-C^{14}. Slices of prostatic tissue obtained from normal and hypoglycemic dogs were incubated for intervals of 30, 60, 90, 120 and 180 min in Ringer-bicarbonate buffer with glucose and 95% O_2-5% CO_2 at 37°. Despite the fact glucose was oxidized, its specific activity did not change appreciably. There were no differences in the incorporation of C^{14} into carbon dioxide and lactate in tissue from normal or hypoglycemic animals. Total radioactivity in the carbon dioxide from glucose-1-C^{14} was significantly higher than that from glucose-6-C^{14} at each time interval. In a series of 3-hr incubations, the effect of lysergic acid diethylamide (LSD) was studied. With 5×10^{-4} M LSD the labeled carbon in carbon dioxide from the glucose-6-C^{14} was markedly increased (85%). The same concentration of LSD had no effect on the incorporation of C^{14} into carbon dioxide from glucose-1-C^{14}. Incorporation of C^{14} into lactate and net lactate production were decreased by 10^{-3} M LSD. These effects were noted in the tissues from both normal and hypoglycemic animals.

479. AUTONOMIC BALANCE IN CARDIAC CONTROL. Robert F. Rushmer. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle.

In intact, unanesthetized dogs, neural reflex mechanisms appear to play an extremely important role in cardiac control. Stimulation of sympathetic nerves to the heart produces changes which resemble those occurring during spontaneous activity or exercise on a treadmill. Alteration in heart rate is a prominent effect produced by stimulating autonomic nerves to the heart. The effects of artificially induced tachycardia on left ventricular performance was studied in intact dogs. In acute experiments, the cardio-acceleration produced by sympathetic cardiac nerves could be precisely overcome by appropriate stimulation of parasympathetic nerves to the heart. With heart rate controlled by an artificial pacemaker, sympathetic nerve stimulation increased ventricular systolic pressure, and reduced the systolic and diastolic dimensions of the left ventricle. Stimulation of vagus nerves to the heart produces little effect on left ventricular performance if the heart rate is controlled. (Supported in part by research grant H-716 from the Natl. Heart Inst., PHS and a grant from the American Heart Assoc.)

480. ACUTE TOXICITY OF ACETYL STROPHANTHIDIN. A. H. Ryan, W. Weil,* J. Spinzia* and S. Soren.* Dept. of Physiology and Pharmacology, Chicago Med. School, Chicago, Ill.

The extreme rapidity with which the full effects of acetyl strophanthidin (AcS) develop and wear off render it suitable for such procedures as ultrarapid digitalization (Gold et al. J. Pharm. & Exper. Therap. 94: 39, 1948) and as a digitalis tolerance test (Lown and Levine, New Eng. J. Med. 250:866, 1954), situations in which full digitalization is usually accomplished within an hour with the possibility of mild toxicity as the end point. Its safety has been questioned because of its speed of action (Purrell and Coggins. Am. Ht. J. 46: 309, 1953). Since the time constant for clearance of AcS could be determined (Ryan, Weil and Spinzia. Fed. Proc. 15:159, 1956) it was possible to determine whether changes occurred in the minimal toxic dose after repeated doses were given. Seventy-five times, cats under pentobarbital received divided doses sufficient to cause extrasystoles two or three times within an hour, without fatality and with recovery without apparent ill effect. Within this period there was no apparent change in the dose required to elicit extrasystoles. When cats were repeatedly brought to the stage of extrasystoles at intervals of 1/2 hr for periods longer than 1-1/2 hr a considerable proportion of animals exhibited extrasystoles after doses smaller than those required earlier. Evidence also indicated that the dose causing extrasystoles moved closer to the lethal dose in such cases. Caution is

indicated in administration of supplemental doses of AcS in the immediate period following ECG evidence of toxicity. No evidence was obtained of unusual toxicity attributable to speed of action. (AcS kindly supplied by Eli Lilly.)

481. CHOLINE DEFICIENCY AND MITOCHONDRIAL MORPHOLOGY IN THE RAT. Simeon Ryan* and Nelson J. Wade. Dept. of Biology, St. Louis Univ., St. Louis, Mo.

Twenty to twenty-five-day old male rats of the St. Louis University strain, weighing 40-45 gm were placed on the following diets: 1) a highly purified choline-deficient diet, 2) the deficient diet plus choline, 3) purina laboratory chow. Mitochondria were studied using the Prichard and Regaud techniques, with modifications. In the renal cortex of the controls filamentous mitochondria predominated. In the renal cortex of the choline-deficient animals the filamentous form was usually lost and granulas mitochondria predominated. In severely damaged areas they frequently disappeared. Deficient animals placed on control diets showed a return to the control picture. Control liver mitochondria were mostly granular or short rod-like in form, densely populated. In the liver of the choline-deficient animal they tended to be smaller and much fewer in number. Deficient animals placed on control diet brought about a good recovery except in areas of residual damage. The classical mitochondrial microscopic morphology appears to be nutritionally vulnerable. The disruptured picture seems to reflect the general pathological condition in liver and kidney and may serve as an index of the physiological state of the cells.

482. PHOSPHATE INTERCHANGES IN TETANIC CONTRACTION OF MUSCLE. Jacob Sacks and Glen J. Dixon.* Dept. of Chemistry, Univ. of Arkansas, Fayetteville.

Cats under pentobarbital anesthesia were injected with tracer phosphate intravenously and 1 hr later one gastrocnemius muscle was subjected to 30 sec tetanus against tension, under essentially anaerobic conditions. The muscle was frozen in situ during continued stimulation and the companion resting muscle then frozen. The acid-soluble phosphorus compounds of both muscles were separated from trichloroacetic acid filtrates by ion exchange chromatography and the relative specific activity of each phosphate group determined after separation by appropriate procedures. The compounds isolated in this way included ADP, ATP, phosphocreatine, glucose-1- and glucose-6- phosphates. No evidence for net breakdown of ATP or accumulation of ADP during the tetanus was found. Isotopic equilibrium between phosphocreatine and the terminal P of ATP was not reached during the tetanus. Nor was isotopic equilibrium reached between the terminal P of ADP and the middle P of ATP. Glucose-1-phosphate and glucose-6-phosphate were not in isotopic equilibrium with each other, nor was glucose-6-phosphate in equilibrium with the terminal P of ATP. Very little AMP was found in the resting muscles; slightly increased amounts were found in the tetanized ones. The amount of lactic acid formed was such that isotopic equilibrium should have been attained between the terminal P of ATP and glucose-6-phosphate if the classical pathway were the mechanism of formation of lactic acid. (Work done under contract AT-(40-1)-1521 between the U.S. Atomic Energy Comm. and the Univ. of Arkansas.)

483. EFFECTS OF GLUCAGON ON METABOLISM OF CARBOHYDRATE AND PROTEIN. James M. Salter,* I. W. F. Davidson* and Charles H. Best. Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Canada.

The profound diabetogenic effect of glucagon in forcefed rats has been reported previously (Salter, Davidson and Best. Fed. Proc. 15:160, 1956). The results of recent studies with D. W. Clarke indicate that the stimulating effect of insulin on glucose uptake in the isolated rat diaphragm is enhanced by pretreatment of the animals with glucagon. These results do not support the view that the glucagon inhibits peripheral utilization of glucose. Glucagon, however, produces an immediate fall in blood amino acids and a large increase in urea excretion. This gluconeogenic action is not eliminated by adrenalectomy but the diabetogenic action of glucagon is supplemented by that of cortisone. When both substances are given weight loss is precipitous and death occurs in from 5-10 days. Removal of the pituitary does not abolish but greatly reduces the catabolic action of glucagon. Preliminary results indicate that the hyperglycemic factor produces a marked increase in nitrogen excretion in human subjects.

484. SODIUM AND POTASSIUM BINDING BY THE MICROSOME FRACTION FROM RAT LIVER CELL HOMOGENATES. Hisashi Sanui* and Nello Pace. Dept. of Physiology, Univ. of California, Berkeley.

The microsome fraction was isolated from rat liver cell homogenates by centrifugation in 0.25 M sucrose and then resuspended in solutions of varying sodium or potassium concentration. The microsomes were equilibrated with the suspending medium for 12 hr, centrifuged and the pellet washed twice with 0.25 M sucrose solution. An qliquot was wet ashed by Vycor glassware and sodium and potassium were determined by flame photometry. Using concentrations of from 0-400 mEq/l. in the bathing medium, a typical saturation type adsorption isotherm was obtained for both sodium and potassium with saturation being reached when the bathing medium contained about 150-200 mEq/l. of either ion. At saturation, about 1.2 mEq of sodium or potassium were bound per gram of microsome nitrogen (or about 16 mEq/l. of microsome pellet). This represents a 15-20 times higher concentration of ions in the pellet than in the last wash solution. The results were reproducible using microsomes isolated from different rats. It was noted that the hemoglobin usually bound by microsomes was displaced by the ions as their concentration in the original equilibrating medium was increased. It appears that the microsome fraction contains specific binding sites capable of attracting sodium or potassium ions. The pH studies indicate some dependent of the ion binding on the hydrogen ion concentration of the suspending medium. The possible significance of these findings in the active transport of ions by the rat liver cell will be discussed. (Supported by Office of Naval Research contract.)

485. EFFECT OF HEMORRHAGE ON DISTRIBUTION OF CARDIAC OUTPUT. Leo A. Sapirstein. Dept. of Physiology, Ohio State Univ., Columbis, Ohio.

Rats were subjected to moderate hemorrhage (25 ml/kg). Five minutes after hemorrhage $K^{42}Cl$ was given by vein; after 30 sec the animals were killed with saturated KCl i.v. The K^{42} content of the individual organs was then determined. Under these conditions the fractionation of the cardiac output is

revealed by the fractional distribution of the isotope (Cir. Research 4:689, 1956). Hemorrhage increases the coronary fraction of the cardiac output to 8.5% from 2.2%; the bronchial fraction increases to 10.4% from 3.3%. The renal fraction falls from 19.4-10.1%. The hepatic and skin fractions are essentially unchanged. All these effects are precisely mimicked by epinephrine (5 gammas/rat); the sympathetic discharge provoked by bilateral carotid occlusion produces only a slight (30%) increase in coronary and bronchial flow fractions and has no effect on the renal fraction. Pitressin (25 milliunits/rat) duplicates the coronary, bronchial and renal effects of hemorrhage; but has the further effect of changing the hepatic flow distribution from one which is predominantly portal to one which is predominantly arterial (normal: portal fraction = 15.9%; hepatic arterial 9.1% : after pitressin : portal fraction = 8.1%; hepatic arterial 25.3%). (Performed as part of a contract between the USAF School of Aviation Medicine, Randolph Field, Tex. and the Ohio State Univ. Research Fndn.)

486. OXYGEN CONSUMPTION OF THE HEART. S. J. Sarnoff, E. Braunwald, R. B. Case,* W. N. Stainsby,* G. H. Welch, Jr.* and R. Macruz.* Lab. of Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

The effects on myocardial O_2 consumption of varying each of the hemodynamic parameters over wide ranges were studied in the isolated, biochemically supported, dog heart preparation (ISH). Unlike previous isolated hearts, this preparation exhibits performance characteristics (pressures, outputs and efficiencies) which are not readily distinguishable from the in situ heart. The O_2 consumption per beat does not exhibit any straightforward relationship either to the stroke work or to filling pressure but depends rather upon the total tension developed, as reflected by the area under the systolic pressure curve. This total tension is determined primarily by peak systolic pressure. Further, at any given peak systolic pressure, myocardial fiber shortening, as reflected by 5-fold changes in stroke volume, appears to have no influence on O_2 consumption except insofar as these changes modify the duration of developed tension; this influence is minimal. A corollary of these data is that, with the heart in any given state, the O_2 utilized per beat is determined by events taking place after the onset of contraction and is not dependent upon the end-diastolic pressure from which the contraction initiated. The possibility that these principles generally apply to contracting muscle will be discussed. An attempt will also be made to interpret the biologic significance of the high efficiencies encountered at the high cardiac outputs.

487. ATROPINE BLOCKADE OF ELECTRICALLY INDUCED HYPOTHALAMIC ACTIVATION OF THE RABBIT ADENOHYPOPHYSIS. Gilbert D. Saul* and Charles H. Sawyer. Univ. of California, Los Angeles and VA Hosp., Long Beach.

To determine whether their blocking actions are exerted at the hypothalamo-hypophyseal level, various drugs known to inhibit "spontaneous" ovulation in the rat and/or copulation-induced ovulation in the rabbit were administered in blocking dosages prior to electrical stimulation of the hypothalamus. The agents included atropine, pentobarbital, morphine, reserpine and SKF-501. Localized bipolar stimulation of the basal tuberal regino with 3.5 v in 5 msec "biphasic" pulses at a frequency of 6/sec in 30-sec on-off sequences for 3- min activated release of pituitary ovulating hormone in 6/7 of the control group. The stimulating electrodes were positioned stereotaxically while employing them as EEG recording electrodes under local anesthesia and their precise locations were subsequently confirmed histologically. Ruptured ovarian follicles at autopsy more than 24 hr after stimulation were indicative of pituitary activation. Atropine sulfate appeared to exert a significant blocking action; 11/15 failed to ovulate. The 4 atropine-treated rabbits that ovulated revealed electrode sites close to the median eminence, i.e. the region of the hypothalamus responding to electrical stimulation was reduced to a small area around the median eminence. Preliminary results indicate that sodium pentobarbital and morphine sulfate block in a similar manner, while reserpine and SKF-501 are ineffective in interrupting direct hypothalamic stimulation of the hypophysis. The former group may be considered as blocking at least in part at the hypothalamic level, while the latter group must function primarily at other sites.

488. NON-UNIFORM DISTRIBUTION OF BLOOD AND GAS IN LUNGS IN PULMONARY DISEASE. George A. Saxton, Jr. and Janet Wolter (intr. by Raymond C. Ingraham). Depts. of Preventive Medicine and Medicine, Univ. of Illinois, Research and Educational Hosps., Chicago.

In an effort to understand blood and gas distribution in diseased lungs and to define the intrinsic pulmonary disorder as precisely as possible, 160 studies have been made on patients with a wide spectrum of pulmonary pathology. The rate of nitrogen washout during breathing of 100% O_2 was followed with a Lilly-type nitrogen meter as an index of uniformity of distribution as inspired gas. The magnitude of the difference between end-tidal alveolar CO_2 and arterial CO_2 was measured as an index of maldistribution of both blood and gas. The results were as follows:

Clinical Types	No. of studies	Av. $P_{a_{CO_2}}$ (mm Hg)	Av. a-A CO_2 diff. (mm Hg)	1 min N_2 conc.
Normals	10	40	1.3	9%
Respir. paralysis (polio)				
Acute, severe involvement	18	33	8	5%
Acute, mod.	37	40	3	14%
Convalescent, mild	25	39	1.5	var
Emphysema, obstruc.	10	55	8	22%
Bronchiectasis	17	45	7	14%
Mixed pul. diseases	43	42	7	var. but none under 8%

These data will be discussed in terms of altered ventilation to perfusion relationships in the lungs in these various disease states.

489. ACTH DISCHARGE FROM THE ADENOHYPOPHYSIS FOLLOWING DECEREBRATION. George Sayers and Marion A. Sayers.* Dept. of Physiology

Western Reserve Univ., Cleveland, Ohio.

Pituitary ACTH activity exhibits "excitatory" and "regressive" phases following application of a noxious stimulus (ether, Pitressin, histamine). The "excitatory" phase is associated with a marked increase in blood ACTH which is demonstrable at 2 min after application of the stimulus and which persists for 10-15 min. Despite continuous action of the noxious agent blood ACTH regresses to pre-stimulus level within 30 min after application of the stimulus. Decerebration (high pontine section) blocks the "excitatory" response to ether, Pitressin or histamine. The results are not in agreement with the thesis that Pitressin contains the corticotropin releasing factor. An extract of ventral hypothalamus (includes median eminence) of calf and epinephrine induce ACTH discharge in the decerebrate rat. Acid (0.1 N HCl or 0.2 N acetic) but not neutral extracts are active. Activity is stable in dilute acid at 100°C for 15 min. The extracts are contaminated with ACTH but the degree of contamination does not account for the marked increase in blood ACTH they induce in decerebrate rats.

490. DEVELOPMENT OF AIR EMBOLISM. K. E. Schaefer, W. McNulty* and Charles Carey.* U.S. Naval Med. Research Lab., New London and Dept. of Pathology, Yale Univ., New Haven, Conn.

Dogs were decompressed within 2 min during which the trachea was closed from a pressure equivalent to 100 and 200 feet to sea level. Recordings of pressure were taken from the abdominal aorta, inferior vena cava, left atrium, pulmonary artery and trachea, intrapleural space and abdominal cavity, using Statham strain gauges and Gauer manometers. All dogs were prepared for the experiment by a prior operation, 2-4 wk before the experiment. Polyethylene cannulae, closed at one end with a thin diaphragm and a flange, were sewed to the pulmonary artery, 2 cm above the valve and to the left auricular appendage. The pressure gradient between intratracheal and intrapleural pressure (transpulmonic pressure) was found to be the decisive factor in producing embolism. Critical level of transpulmonic pressure = 60-70 mm Hg. Binders around chest and abdomen prevent distention of the lungs and keep the transpulmonic pressure from reaching a critical value. Abdominal binders fail to give a protection against air embolism. In every case of air embolism bubbles were found in the carotid artery bubble trap. A transient circulatory failure indicated in the fact, that all circulatory pressures in the pulmonary as well as the systemic circulation approach the same level with a simultaneous disappearance of pulse pressure occurred whether or not air embolism developed—and can therefore not be the cause of air embolism.

491. SIMULTANEOUS CINERADIOGRAPHIC AND KYMOGRAPHIC STUDY OF HUMAN GASTRIC ANTRAL MOTILITY (MOTION PICTURE). Jerry F. Schlegel, Alan W. M. Smith and Charles F. Code (intr. by Hiram E. Essex). Mayo Fndn. and Mayo Clinic, Rochester, Minn.

Simultaneous cineradiographic and balloon photokymographic records of gastric antral motility have been obtained in 19 health persons. Both type I and type II waves in the balloon kymographic records have been shown to be annular contractions which invariably progress over the antrum toward the pylorus in a peristaltic manner. The 2 types of waves differ mainly in size in both balloon and cineradiographic recordings. The type I waves in the balloon kymographic records are due to small annular constrictions produced apparently by contraction of only a part of the circularly disposed muscle fibers of the gastric wall. Type II waves in the balloon records are, on the other hand, due to annular constructions of the entire thickness of the gastric wall, presumably produced by powerful contractions of most, if not all, of the circularly disposed muscle fibers of the gastric wall. About 70% of type II waves propelled barium sulfate through the pylorus into the duodenum, while only 17% of the type I waves exerted this action. It is concluded that the major function of type II contractions is propulsion, while their secondary functions is mixing and that mixing is the primary action of the type I contractions and propulsion is secondary. Failure of barium sulfate to pass through the pylorus when the contraction passes over the antrum occurred either when the pylorus failed to relax or when the contraction faded out just before reaching the pylorus.

492. UREA EXCRETION IN SHEEP AND DOG. Bodil Schmidt-Nielsen, H. V. Murdaugh,* H. Osaki* and Roberta O'Dell.* Duke Univ., Durham, N. C.

In order to elucidate the renal mechanism for urea excretion we studied the effect on urea clearance of diet and urine flow. Previous studies showed that the camel (a ruminant) during low protein intake can conserve urea to the point that only 1% of the filtered urea is excreted in the urine; this can be related to the fact that ruminants can utilize urea for protein synthesis. In another ruminant, the sheep, the urea/inulin clearance ratio decreased during low protein regime similarly asin the camel; the same was found in dogs but less pronounced. Acute nitrogen loads given during low protein regime increased the urea clearance in the dog but decreased it further in the sheep. The diuresis affected the fraction of filtered urea excreted in a very characteristic manner in both sheep and dog. At low urine flows there was a marked difference in this fraction between animals on low and normal protein diets. However, at higher urine flows the difference dimished, and at extreme flows the fraction excreted was the same on both diets. Two alternate interpretations can be offered for the difference in urea clearance on various nitrogen intakes: a) the permeability of the tubules to urea can change in a selective manner with nitrogen intake; b) a regulated tubular secretion of urea is responsible for the differences. To explain the diminishing difference at higher urine flows we must assume that such a secretion is graded according to urine flow. (Supported by American Heart Assoc. and by the Natl. Insts. of Health Grant H-2228.)

493. SECRETION OF HYPERTONIC SOLUTIONS IN MARINE BIRDS. Knut Schmidt-Nielsen, C. B. Jörgensen,* and H. Osaki.* Duke Univ., Durham, N. C.

It has often been suggested that marine birds drink sea water to cover their normal needs for water. This problem was investigated in young cormorants (Phalacrocorax auritus). When nestlings and young adults in captivity were fed fresh fish, the water content of the food was much higher than necessary for the renal excretion of the salts of the food. Birds feeding on marine fish therefore seem independent of drinking water and ingestion of sea water would impair an otherwise favorable water and salt balance. However, during experimental salt loads cormorants react in an unexpected way. Under such load they secrete a highly hypertonic fluid from the nose, the secretion dripping out from the internal nares and collecting at the tip of the beak, from which the drops are shaken with a sudden jerk of the head. Concentration (500-600 mN NaCl) and rate of flow (up to

0.15 ml/min in a 1.5-kg bird) are so high that with continuous secretion the entire NaCl content of the body could be eliminated in roughly 10 hr. The fluid contains practically only sodium and chloride in nearly equivalent amounts. Production of the nasal secretion is also stimulated by non-electrolytic osmotic load (sucrose). The secretion obtained in response to such stimulation is similar to that obtained by stimulation with NaCl, indicating that the mechanism responds to general osmotic conditions, rather than specifically to NaCl concentration. (Supported by the Natl. Insts. of Health Grant #H-2228.)

494. NOREPINEPHRINE: EFFECTS ON LEFT VENTRICULAR WORK, O_2 CONSUMPTION, SODIUM AND POTASSIUM EXCHANGE. J. E. Schmitthenner,* I. Forte,* T. Shakashir,* Cecilia Riegel* and J. H. Hafkenschiel. Lankenau Hosp., Philadelphia, Pa.

In dogs anesthetized with pentobarbital, measurements were made of stroke volume (Fick), cardiac rate, mean arterial pressure, limb lead electrocardiogram, coronary blood flow (nitrous oxide desaturation) and hemoglobin, hematocrit, pH, glucose, lactate, pyruvate, oxygen content, sodium and potassium concentration in arterial and coronary sinus blood. The effects of norepinephrine infusion, 1 μg/kg/min, were compared with those of control saline infusion. Norepinephrine produced elevated arterial pressure and left ventricular work, with greater increase in stroke work as heart rate slowed. Coronary blood flow was increased. Hemoglobin and hematocrit concentrations were raised, with more oxygen extracted from arterial blood, so that the increase in left ventricular oxygen consumption exceeded that of coronary blood flow. Respiratory minute volume and total oxygen consumption were increased. Left ventricular efficiency was maintained and myocardial usage of carbohydrate unchanged. Although T wave amplitude was augmented by norepinephrine, the extracellular blood concentration of sodium and potassium ions (when corrected for hemoconcentration) was reduced. During norepinephrine infusion potassium ion concentration of coronary sinus blood fell further, suggesting a decrease in myocardial extracellular potassium loss with each systole. This is consistent with the thesis that norepinephrine has a direct action on the myocardial muscle cell membrane, allowing for a change in the usual migration of ions during contraction. (Supported by grants from the Natl. Heart Inst., PHS, H1817, HF6498 and Tobacco Industry Research Committee OH-171.)

495. INCREASE IN AMYLASE OF RAT SUBMAXILLARY GLAND IN RESPONSE TO FEEDING OR ACETYLCHOLINE. L. H. Schneyer and C. A. Schneyer.* Depts. of Physiology and Clin. Dentistry, Univ. of Alabama Med. Ctr., Birmingham.

Stimulation of rat submaxillary-sublingual blands by subcutaneous administration of pilocarpine has been shown to result in a rapid increase in the level of gland amylase without prior depletion of the enzyme or accompanying appreciable change in gland wet weight (Schneyer and Schneyer. Am. J. Physiol., in press). Investigation of this phenomenon has now been extended to include the separate effects of acetylcholine and feeding. It has been found that in each of these conditions there results, in adequately fasted animals, a significant and rapid increase in gland amylase; this increase may be suppressed by simultaneous administration of atropine. These findings are consistent with the thesis that parasympathetic stimulation exerts, independently of enzyme depletion, an effect on the process of enzyme synthesis. (Supported by a research grant, RG-4841, from the Natl. Insts. of Health.)

496. PENTOTHAL BLOCK OF SINGLE NERVE FIBERS AND SUBSEQUENT REVIVAL BY MEANS OF ANODAL POLARIZATION. Gordon M. Schoepfle. Dept. of Physiology. Washington Univ. School of Medicine, St. Louis, Mo.

Single fibers of the frog sciatic nerve can be rendered completely inexcitable to cathodal stimuli by a concentration of sodium pentothal (0.2%) which has no perceptible effect on either membrane potential or membrane resistance. Procaine in a 1% concentration produces identical effects. However, the pentothal-depressed fiber shows complete recovery if stimulated during a period of anodal polarization. Responses of this preparation at various intervals subsequent to a single stepwise rediction in the anodal polarization show a decline in spike height which is an exponential function of this interval. The time parameter of this decline is identical with that obtained on break of anodal polarization in the normal fiber. In terms of the Hodgkin-Huxley scheme this would imply that pentothal brings about enhancement of sodium inactivation in the absence of any change in resting membrane potential. An intrinsic time parameter of the inactivation process is unchanged.

497. ELECTRIC ACTIVITY AND ION TRANSPORT WITH SINGLE ELECTROPLAX. Ernest Schoffeniels (intr. by David Nachmansohn). College of Physicians and Surgeons, Columbia Univ., New York City.

Following the pattern of Ussing's frog skin preparation for investigating ion transport, a single electroplax of Electrophorus electricus is being used for studies of chemical and physical factors affecting the permeability of the conducting and non-conducting membrane of this cell. Effects of a number of tertiary and quaternary nitrogen derivatives known to affect the acetylcholine system have been tested. All compounds penetrate into the cell interior. An important progress over previously reported studies with electroplax is the reversibility of these effects except for decamethonium. The depolarizing action of quaternary compounds, such as acetylcholine, carbamylcholine, prostigmine, must be attributed to the effect on the acetylcholine receptors at the multiple synaptic junctions. This has been demonstrated by the protecting action of small concentrations of curare or eserine. Only tertiary compounds such as eserine, the tertiary analogue of prostigmine, procain and alkylphosphates (DFP, Tabun) affect the propagated spike elicited directly. This modifies the previous assumption of a direct action of quaternary compounds on the conducting membrane of electroplax which thus seems to be similarly protected against quaternary nitrogen derivatives as the membrane of axons and muscle fibers. If the contact between DFP and electroplax exceeds half an hour, the block of electric activity becomes irreversible, comparable to the slowly progressive inhibition of acetylcholinesterase as a function of time. Effects of chemical compounds and changes in ionic environment upon resting potential and electric activity will be discussed in the light of recent views on ion transport and membrane permeability.

498. METABOLIC ACCLIMATION TO COLD IN MAN. P. F. Scholander, H. T. Hammel,* K. Lange Andersen* and Y. Loyning.* Univ. Inst. of Zoophysiology, and Physiology Dept., Univ. of Pennsylvania,

Norwegian Air Force Inst. of Aviation Medicine and Navy Med. Office, Oslo, Norway.

In September and October 8 men lived out in the Norwegian mountains above tree line in essentially summer clothing and with insufficient night protection. Snow and sleet were common and night temperatures were usually between 0 and +5°C. The men had enough food and kept busy hiking, fishing and hunting. The nights were spent underneath a tarpaulin rigged as a rainproof shelter with one side open. The subjects slept, naked except for socks and shorts, in a single blanket sleeping bag with a hydrophobic cover. After 6 wk in the field they had acquired a considerable acclimation. Tests showed that they then stayed warm from head to foot all night and slept well. Heat production remained 50-60% higher than the basal all night and while they were asleep. Shivering, visible or detectable by electromyography, occurred frequently during sleep. Control subjects had a less elevated metabolic rate and were unable to rest and sleep due to chilling, especially of the feet. In the acclimated men, therefore, an increased heat production alleviated the shell cooling from which unacclimated people suffer. When bicycling naked in the cold, just enough to maintain the rectal temperature, the cold-acclimated men used as much oxygen as the controls, so neither during exercise nor during rest did acclimation result in increased insulation by shell cooling. Metabolic acclimation to cold is well known in other homeotherms.

499. PRIMARY CORTICAL RESPONSES EVOKED BY STIMULATION OF LATERAL GENICULATE RADIATIONS IN UNANESTHETIZED CATS. Arnold Schulman and Edward V. Evarts (intr. by Wade H. Marshall). Natl. Inst. of Mental Health, Bethesda, Md.

Cortical responses to near-maximal stimulation of lateral geniculate radiations were recorded in unanesthetized cats with chronically implanted macroelectrodes, in the same preparations under pentobarbital anesthesia and in cats decerebrated by mid-collicular section. The configuration of responses to single radiation shocks was similar in decerebrate and unanesthetized preparations. Responses in these preparations differed slightly from responses evoked in anesthetized preparations, the latter generally failing to show one or more discontinuities which consistently appeared on the descending portion of the final positive component of responses in decerebrate and unanesthetized cats. Decerebrate and unanesthetized preparations also differed from anesthetized preparations with respect to the recovery cycles of evoked responses. Observations were obtained on interaction between responses to paired shocks delivered to geniculate radiations at intervals from 3.2-1000 msec. In decerebrate and unanesthetized preparations, subnormality of responses to the test stimulus developed gradually, reaching maximum at pair separations of 20 msec. In anesthetized preparations, maximum subnormality was apparent at the smallest pair separations studied. Beyond 20 msec, major differences in the recovery cycles of all 3 types of preparations were not observed. Observations were obtained concerning the interaction between responses to retinal photic stimulation and stimulation of geniculate radiations. Facilitation of responses to radiation stimulation occurred when photic stimuli preceded radiation stimuli by 20 to 30 msec.

500. A POLYSACCHARIDE OBTAINED FROM PREPARATIONS OF ISOLATED CALF THYMUS NUCLEI. Irving L. Schwartz and Shinji Itoh.* Rockefeller Inst. for Med. Research, New York City.

In the course of studies of the binding of cations to intracellular components, it was observed that preparations of isolated nuclei contained a polysaccharide which appeared to be correlated with the nuclear sodium content. The isolated nuclei, as well as the fresh whole tissues from which they were derived, were treated by standard methods to hydrolyze protein and depolymerize nucleic acid and then dialyzed against distilled water for 24 hr at 2°C. The resulting solution was shaken with chloroform and amyl alcohol (100 cc solution: 20 cc chloroform: 7 cc amyl alcohol) for 24 hr at 2°C. The aqueous phase was passed through a column of kaolin. Three volumes of 95% ethanol saturated with sodium chloride was added to the effluent; the resulting precipitate was recovered by centrifugation, dried and dissolved in distilled water. The material in solution contained approximately equal parts of uronic acid and hexosamine. It resembled chondroitin sulfate in its electrophoretic mobility on paper (acetate buffer, pH 5.2, $\mu = 0.1$), and in its mobility on paper chromatography (25% propanol in M/15 phosphate buffer, pH 6.5). However, chromatography on a column of Dowex 50 showed that its principal hexosamine was glucosamein, not galactosamine. (Support of Dr. Itoh by the Dazian Fndn. for Med. Research and the Helen Hay Whitney Fndn. is gratefully acknowledged.)

501. SICKLING OF ERYTHROCYTES WITH I-A ELECTROPHORETIC HEMOGLOBIN PATTERN. I. R. Schwartz, Jean Atwater, Evalyn Repplinger and L. M. Tocantins. (intr. by W. J. Snape). Cardeza Fndn., Jefferson Med. College, Philadelphia, Pa.

A 25-year-old woman (J. W.) whose red cells sickled with metabisulfite had mild anemia and no other abnormalities. Paper electrophoresis (pH 8.6) of hemoglobin hemolysates produced 2 distinct hemoglobin types, a smaller component of A mobility and a larger faster moving component. Freezing and thawing did not alter the pattern. At pH 6.5, both components migrated cathodally, but normal hemoglobin moved faster. The fast component had the same mobility as known hemoglobin I (Rucknagel. Blood 1955). No hemoglobin S was present. One minute alkali denaturation value was 0.5% and ferrohemoglobin solubility (Goldberg, Clin. Chem., in press), was 86% (normal:over 90%). Sickling was usually positive with 2% and consistently strongly positive after an hour with 4% sodium metabisulfite and acid bisulfite, but negative in simple sealed preparations, with ascorbic acid and after CO_2 saturation. Concentrated hemoglobin solutions treated with CO_2 (Singer. Blood 1953) did not gell. The patient's father, deceased, was half American Indian and half white (Irish). J. W. is his only surviving kin. Erythrocytes of the mother (Negro), 2 half siblings and 1 child were normal or elevated in number and markedly hypochromic; another child and 6 half-siblings had similar but milder changes. In the relatives, no abnormal hemoglobins or sickling tendency were found, target cells were rare and, except in 1 child, fetal hemoglobin was absent. J. W. probably inherited hemoglobin-I from the father and thalassemia trait from the mother and may owe her large preponderance of abnormal hemoglobin to an I-thalassemia combination.

502. EVIDENCE FOR A TWO-STAGE PROCESS IN THE NORMAL REABSORPTION OF BICARBONATE. William B. Schwartz, Adrien J. Falbriard and Arnold S. Relman (intr. by E. R. Loew). Depts. of Medicine, Tufts Univ. School of Medicine and

Boston Univ. School of Medicine, Boston, Mass.

In a previous report the relationship between plasma bicarbonate concentration and renal response to acetazoleamide (10-40 mg/kg) was described. The curve relating plasma level to bicarbonate reabsorption following administration of the carbonic anhydrase inhibitor was found to resemble the Michaelis-Menten relationship between substrate concentration and enzyme activity, suggesting that when enzyme activity is partially inhibited the enzymatic reaction is the rate-limiting step in the overall process of bicarbonate reabsorption. In the present studies the relationship between plasma level and bicarbonate reabsorption was defined for 2 other doses of inhibitor (1 mg/kg and 5 mg/kg) and compared to the original results with the larger dose. A family of 3 similar curves was obtained, all apparently obeying the Michaelis-Menten formulation. This suggestion is supported by a double-reciprocal plot (Lineweaver-Burk) which yields a series of straight lines resembling the effect of increasing doses of a non-competitive inhibitor on the rate of an enzymatic reaction. It is concluded that normally, at plasma levels below threshold, some first-order process (not an enzymatic reaction) is the limiting step in bicarbonate reabsorption; the leveling-off of the curve of reabsorption when the bicarbonate "T_m" is reached probably represents the point at which the "V_{max}" for the enzyme becomes the rate-limiting step.

503. ACTIVITY IN CUTANEOUS SENSORY NERVES IN RESPONSE TO STIMULATION OF SKIN WITH RADIANT ENERGY. D. Scott Jr. and James Daniel Hardy. Dept. of Physiology, Univ. of Pennsylvania, School of Medicine, Philadelphia.

While the response of sensory endings in the skin to thermal stimulation has been previously studied by other workers, this response has not been related to exact skin temperature as determined by radiometric methods. The latter measure has been applied extensively to the subjective sensations of warmth and pain in man and animals. To bridge the gap between these two types of studies the technique of recording activity from sensory nerve fibers in a few fiber preparation obtained from a dorsal cutaneous nerve of the rat has been employed during stimulation of the skin with radiant energy at known rates. Simultaneous skin temperature measurements were obtained by radiometer and a direct relationship obtained between the type of discharge recorded from afferent nerve fibers and the temperature of the skin. Since the quality of cutaneous sensation that will be aroused at a given skin temperature is known, a correlation can now be made between sensation and the type of nerve fibers which carry this message. Especial interest attaches to the character of the afferent activity recorded at skin temperatures just below and just above 45°C, as skin temperatures in excess of this value are associated with the sensation of pain. (Aided in part by a grant from the Office of Naval Research N-onr-551 (11).)

504. EFFECTS OF "TOTAL" PANCREATECTOMY IN THE RAT. Robert O. Scow. Inst. de Biologia y Medicina Exptl., Buenos Aires, Argentina, and Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

A method for removing more than 99.5% of the pancreas has been developed. At 19 hr postoperative the rats had hyperglycemia, glycosuria, ketonuria, gastric retention of fluid and large amounts of fat in the liver and the renal tubules (intracellular). Within 48 hr after pancreatectomy, the rats died in diabetic coma if not treated with insulin. Large amounts of insulin were needed by these rats when food was given (always tube-fed)—2.5 units of regular insulin/gm of diet. When fasted, they were sensitive to very small amounts of insulin (3.3 milliunits lowered blood glucose 40 mg/100 ml in 1 hr). Intestinal absorption of nitrogen was greatly impaired after the removal of more than 99% of the pancreas, whereas it was normal when 5% of the gland was left. Feeding pancreatin, 10 mg/gm of diet, restored absorption to normal. Pancreatectomized rats given insulin and pancreatin have survived more than 6 months. They died in diabetic coma within 48 hr after stopping insulin therapy.

505. SPHINGOSINE AND INHIBITION OF CONVERSION OF PROTHROMBIN TO THROMBIN WITH THREONE OR THROMBOPLASTIN. Walter H. Seegers, Ricardo H. Landaburu* and E. Hecht.* Dept. of Physiology and Pharmacology, Wayne State Univ., College of Medicine, Detroit, Mich.

When purified platelet cofactor I and purified platelet factor 3 are together in solution, the activity is by definition threone activity. For the conversion of purified prothrombin to thrombin, calcium ions, Ac-globulin and threone can be used. The yield of thrombin may be completely inhibited with either synthetic sphingosine or sphingosine concentrated from natural sources. In low concentration, sphingosine tends to accelerate the interactions rather than inhibit them. The inhibition of prothrombin activation can by overcome either with large amounts of purified platelet factor 3 or with large amounts of purified platelet cofactor I. Sphingosine also inhibits the conversion of purified prothrombin to thrombin with calcium ions, Ac-globulin and lung extract thromboplastin. In high concentration, the thromboplastin overcomes the retarding effect of sphingosine.

506. HEMODYNAMICS OF INTESTINAL CIRCULATION. Ewald E. Selkurt, Mario P. Scibetta* and Theodore E. Cull.* Dept. of Physiology, Western Reserve School of Medicine, Cleveland, Ohio.

Recent investigation of the role of the intestinal circulation in hemorrhagic shock has stimulated further research on this vascular bed. This report concerns some of the fundamental hemodynamic characteristics of the intestinal circulation, as analyzed in a segment of isolated and denervated intestinal loop of the dog. Flow was varied through a wide range of perfusion pressures. Simultaneous measurement of inflow and outflow showed that a minimum of ca 30 sec was necessary for flow equilibrium to be attained after each change in pressure. Therefore, flow values obtained 1-4 min were averaged in construction of a pressure-flow curve. The mean curve so obtained was convex toward the pressure axis. Control flow averaged 22.6 cc/min at an average pressure of 119 mm Hg. Zero flow occurred at an average of 10 mm Hg. At 240 mm, flow averaged 57 cc/min/100 gm of intestine. Calculated vascular resistance progressively decreased as pressure was increased above control. Conversely, when pressure was decreased vascular resistance increased, more rapidly below 80 mm, reaching infinity at 10 mm. The simplest explanation for the pressure-flow relationship appeared to be that the diameter of the arteries and arterioles varied with intralumenal pressure, so that vascular resistance varied inversely to the cross-sectional area. Interesting deviations from the typical pattern will be discussed, as well as application of the pressure-flow curve to analysis of vascular resistance changes.

507. EFFECT OF SPINAL-CORD TRANSECTION UPON SKELETAL CHANGES INDUCED BY AMINOACETONITRILE (AAN). Hans Selye and R. K. Mishra.* Inst. of Exptl. Medicine and Surgery, Univ. of Montreal, Montreal, Canada.

Forty female Sprague-Dawley rats (average body weight of 101 gm) were subdivided into 4 equal groups: 1) untreated controls; 2) 5 mg of aminoacetonitrile hydrosulfate twice daily subcutaneously; 3) transection of the spinal cord between 10th and 11th thoracic vertebrae; 4) AAN treatment (as in group 2) plus transection of the spinal cord (as in group 3). Autopsy, on 16th day, showed that AAN produced marked bone-lathyrism (multiple exostoses, hypertrophy of junction-cartilage plates, slipped epiphyses) in the intact but not in the spinal-cord-transected rats. Even histologically, bone lesions were hardly detectable after spinal-cord transection. AAN produced abundant periosteal new-bone formation and "basophilic bone globules" (typical of experimental lathyrism) in the intact but not in the spinal-cord transected animals. The production by AAN of sub-periosteal hemorrhages and junction-cartilage lesions was not prevented by spinal-cord transection and, frequently, foci of cartilage were found free within sub-periosteal hemorrhages and even in adjacent veins. The muscle atrophy induced by spinal-cord transection was greatly enhanced by concurrent administration of AAN. Spinal-cord transection, without AAN treatment, induced osteitis-fibrosa-like bone absorption, with excessive development of osteoclasts. (Supported by the Muscular Dystrophy Assoc. of Canada.)

508. EXPERIMENTAL MUSCULAR DYSTROPHY IN THE DUTCH RABBIT. L. C. Senay, Jr.* and H. M. Hines. Dept. of Physiology, State Univ. of Iowa, Iowa City.

Isometric tetanus tensions and fatigue rates were recorded from slips of diaphragm from normal rabbits, rabbits injected with 1 or 2 basic toxin doses (1/4 mld/kg of diphtheria toxin), and rabbits injected with neutralized toxin. The animals receiving 1 dose were sacrificed at 12 and 24 hr following the toxin injection, while those receiving 2 doses in a 4-day period were allowed to live either for 6 or 21 days after the first toxin injection. Faster fatigue rates and lower maximum isometric tension values were consistently seen at 6 days, whereas results at 12 hr, 24 hr and 21 days were somewhat inconsistent. The effect on the diaphragm of other dystrophy producing agents such as plasmocid was investigated. In studies on the maximum isometric tetanus tension/gm and fatigue rates of the gastrocnemii, 1, 2 and 3 injections of the basic toxin dosage were given over a 5-day period. The gastrocnemii from animals injected with the toxin fatigued faster than those of the control but no difference was noted in the isometric tetanus tension. Adrenal weights (mg/100 gm body weight) in all 3 categories were significantly greater than the control weights but among the 3 experimental groups no difference in the adrenal weights was noted. Histological studies revealed damage of from 10%-80% of the muscle fibers in the diaphragms of the 6-day animals while the gastrocnemii of similar animals rarely had damage exceeding 1-2% of the inspected fibers.

509. TRANSLOCATION OF FLUID FROM PERFUSED HINDLIMB OF THE DOG. O. W. Shadle, M. Zuckoff* and J. N. Diana.* Physiology Dept., Univ. of Louisville School of Medicine, Louisville, Ky.

Blood is translocated from the dog's systemic vessels into the pulmonary vessels during the intravenous infusion of l-arterenol. To obtain more information on the mechanism of this phenomenon we have studied the effect of continuous l-arterenol infusion on the isolated, perfused hindlimb of the dog. Continuous records were made of arterial and small vein pressures, hindlimb weight and venous outflow, before and during each drug infusion. As the drug first reached the limb there was generally an increase in limb weight, accompanying a rise in arterial pressure and a fall in small vein pressure and venous outflow. This initial weight gain was attributed to a blood volume increase in the arteries. Following the initial increase, the hindlimb lost weight, the small vein pressure increased, arterial pressure remained constant and venous outflow from the limb exceeded arterial inflow. This weight loss was interpreted as a translocation of blood brought about by venous constriction. To eliminate the possibility that these weight changes were due to diffusion across the capillary membrane, the perfused red cells were "tagged" with radiophosphate (P^{*32}) and the plasma proteins were "tagged" with T-1824. Transcapillary diffusion acted to oppose the changes in gross limb weight observed. (Supported by grants from the Louisville and Kentucky Heart Assocs. and the American Heart Inst.)

510. HUMAN SALIVA: VOLUME, SODIUM, POTASSIUM AND Na/K. Ira L. Shannon (intr. by: Herman I. Chinn). Dept. of Exptl. Dentistry, School of Aviation Medicine, USAF, Randolph AFB, Texas.

Five-minute saliva samples were collected from 402 males and 60 females, under the stimulus of one small rubber band. Similar sampling at 11:00 a.m. (350 males) and at 3:00 p.m. (184 males). Mean volume for males (4.594ml) was more than double that for females (2.230 ml). Mean potassium was: males - 24.380 mEq/l. (S.D. 3.48); females - 25.040 mEq/l. (S.D. 4.040). Potassium and flow rate were negatively correlated (r = -.41). Positive correlation (r = .27) was noted between sodium and volume. Mean sodium: males - 13.205 mEq/l. (S.D. 7.064); females - 13.500 mEq/l. (S.D. 6.046). Na/K followed sodium pattern. The male mean was .5589 (S.D. .336); females - .550 (S.D. .266). Na/K:volume correlation coefficient was r = .42. Fifty-one male subjects were sampled at 2-hr intervals from 5:00 a.m. until 9:00 p.m. No predictable diurnal pattern could be discerned at this low rate of flow. Sodium levels were consistently higher on arising than at any other time of day. Sampling was done (9:00 a.m.) of 538 males and 102 females stimulated by 3 large rubber bands. Correction formulae adjusting all flow rates to 2 ml/min brought the sodium and Na/K for the 2 types of stimulation into very close agreement.

511. OCULOMOTOR PATHWAYS IN BRAIN STEM AS OUTLINED BY ELECTRICAL STIMULATION. S. Shanzer,* C. A. Papatheodorou,* I. H. Wagman and M. B. Bender. Dept. of Neurology, Mount Sinai Hosp., New York City.

The brain stem was systematically explored by electrical stimulation in 13 alert cervically transected monkeys mounted in a stereotaxic instrument. Most of the elicited conjugate eye movements were horizontal, either ipsilateral or contralateral. Oblique movements were frequently observed. The frequency of these responses differ from that obtained in anesthetized monkeys, in which eye centering and horizontal deviations are common, while oblique and vertical movements are rare. Contralateral ocular deviations were obtained on stimulation of pretectal region and tegmentum of the midbrain, both in the paramedian and lateral zones. The posterior extent corresponded

approximately to the border between midbrain and pons. No contralateral movements were obtained ventrally to the level of the 3rd nerve nucleus. Ipsilateral movements were elicited from the paramedian reticular formation caudally and ventrally to the 3rd nerve nucleus and extended caudally beyond the level of the abducens nucleus into the medulla. The lateral extent was 3 mm from the median raphé. These results indicate that crossing of the pathways takes place in a fairly extensive area between the levels of the 3rd and 4th nerve nuclei. (Aided in part by USPHS grant B-294.)

512. ADRENAL CORTEX AND POST-TRAUMATIC SODIUM AND POTASSIUM METABOLISM. Leonard Share and John B. Stadler.* Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Experiments were conducted in an attempt to clarify the role of the adrenal cortex in the metabolic changes which follow trauma. Adrenalectomized or sham adrenalectomized male rats weighing 200-500 gm were maintained by force-feeding a diet containing supplemental sodium chloride. They were allowed ad lib access to distilled water and were kept in metabolism cages for the collection of urine. Following several days of control urine collection, femurs, tibias and fibulas of both hind legs were fractured under ether anesthesia. The changes in the urinary excretion of sodium and potassium by adrenalectomized and sham adrenalectomized rats were qualitatively similar. Sodium was retained on the first post-fracture day. This was more marked in the adrenalectomized group. There was an increased urinary loss of potassium from the second to about the 8th post-fracture days. More potassium was lost by the sham adrenalectomized rats. Analysis of the electrolyte content of the hind leg muscle and bone from fractured adrenalectomized rats indicated that the negative potassium balance could be accounted for only in part by potassium loss from injured tissue. The plasma potassium concentration was significantly elevated on the first 1 or 2 days after fracture in both groups of rats. It is concluded that the presence of the adrenal cortex is not essential for the changes in sodium and potassium metabolism which follow trauma. However, its presence does quantitatively modify these changes. (Supported by research grant N-1640 (C3), Natl. Heart Inst., PHS.)

513. EFFECT OF SEROTONIN AND RESERPINE ON INTERDIGESTIVE GASTRIC SECRETION IN THE RAT. Harry Shay, David C. H. Sun* and Margot Gruenstein.* Fels Research Inst., Temple Univ. Med. Ctr., Philadelphia, Pa.

Serotonin has been shown to increase gastrointestinal motility. Present studies were done to evaluate effect of serotonin on gastric secretion using the rat preparation developed in our laboratory. Animals weighing 120-200 gm were used. In all experiments litter mates served as control and treated animals. Six groups of experiments were done with serotonin given at 6-dose levels in ascending log interval, ranging from 0.0625-2.0 mg/100-gm body weight. Each group consists of at least 20 animals for the control and 20 for the drug study. After proper starvation the pylorus was ligated and the stomach lavaged with saline and emptied completely. Serotonin was then given subcutaneously. Animals were inspected at frequent intervals for gross behavior and signs of toxicity. Four hours after drug administration, animals were sacrificed and their gastric content collected quantitatively. These were measured and analyzed for free and total acidity, pH and pepsin concentration when enough material was available. The results show that serotonin, in doses of 1 or 2 mg/100 gm, produced a significant depression in volume and acid output of gastric secretion. At lower dosage level no significant difference in volume and acid output between control and drug studies occurred. The above results will be compared with a similar study done with reserpineand the possible significance of these in emotional stress induced gastric secretion will be considered.

514. CATHETER ARTIFACTS IN INDICATOR DILUTION EXPERIMENTS. C. W. Sheppard. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

In the Steward-Hamilton method of determining cardiac output and other similar procedures, analysis of the dye transients suffers from the smearing of the recorded curves, caused by laminar flow in the catheter through which samples are taken. The effect has been analyzed by numerical integration using the formula for dye dispersion in a uniform tube (Sheppard, Minnesota Med. 37:93) and applying it to curves of the random walk type. The distortion is small if the ratio of catheter volume to the volume of the circulatory labyrinth is small. Serious distortion occurs for ratios of 0.1 or larger and significant effects may occur at smaller ratios depending on the required precision of the experiment. Alterations in effective volume of the system relative to its true volume occur and changes in the randomizing constant are reflected in changes in the sharpness of the peak. Effective zero time can be displaced from the true value by a horizontal shifting of the entire curve. For randomizing constants in the neighborhood of 0.5, such as are encountered in fitting curves to available data, the principal effect is a change in the effective system volume. In such cases correction can be made with fair precision by adding to the bed volume the catheter volume times 1.5.

515. CAPILLARY PERMEABILITY TO MACROMOLECULES: STRETCHED PORE PHENOMENON. H. H. Shirley, Jr.,* C. G. Wolfram,* K. Wasserman and H. S. Mayerson. Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.

Radioactive iodinated serum albumin and dextran fractions of average molecular weights of 51,000-255,000 are injected into nembutalized dogs and concentration changes of these substances are followed in plasma and thoracic and right duct lymphs for 4-6 hr. At this time, when a "steady state" has been established between plasma and lymph, the plasma volumes of the dogs are increased by the infusion of 40 ml/kg of 5% serum albumin in 0.9% saline solution. This results in a significant and striking increase in the concentrations of the injected radioactive iodinated albumin and dextrans in right duct and thoracic duct lymph in spite of increased lymph flows. The increase in dextran lymph/plasma concentration ratios occurs with all molecular weight fractions. These results are interpreted as re-affirming our previously formulated concept that infusions producing plasma volume expansion decrease the resistance of the capillary wall to the passage of macromolecules or increase the size of the capillary "pores." The concept of capillary permeability as a function of "pore" size must, therefore, be modified to include a labile capillary "pore" size, subject to change with variations in plasma volume. (Supported by a grant from the Med. Research and Development Div., Office of the Surgeon General, Dept. of the Army, under Contract DA-49-007-MD-38.)

516. DIFFUSING CAPACITY AND AREA OF ALVEOLAR CAPILLARY BED. A. A. Siebens, N. R. Frank,* R. A. Rauf* and Louise Ray.* Dept. of Physiology, State Univ. of New York, Downstate Med. Ctr. and Cardiopulmonary Lab., St. Albans Naval Hospital, New York City.

Diffusing capacities following pulmonary resection for bronchiectasis are lowered roughly in proportion to the magnitude of resection, an observation interpreted to suggest that this measurement is in fact a function of the area of the alveolar capillary bed (Am. J. Physiol. 179:673, 1954). This abstract concerns two possible objections to this interpretation in addition to measurements of the dog's diffusing capacity relevant to the argument. a) It is conceivable that bronchiectasis does itself lower the diffusing capacity independently of resection. However, only 2 of 7 preoperative patients with cronchiectasis, 4 of whom had bilateral disease, have demonstrated abnormally low diffusing capacities. b) Intrathoracic procedures in themselves might also somehow reduce diffusing capacity. That this is an unlikely objection is suggested both by increase in diffusing capacity among patients who had procedures (decortication, excision of large bleb) releasing confined lung as well as by preoperative reductions in diffusing capacity among patients demonstrating impaired unilateral oxygen uptake. c) Eleven measurements of diffusing capacity were made in 5 dogs weighing 37-50 lb. Metabolic rates were 5-7 times resting levels. The lowest diffusing capacity in the smallest dog was 13 and the highest value in the heaviest dog was 25. Four measurements in two 50-lb dogs varied from 19-25. These are figures one would predict if dog lung's diffusing surface differs from adult man's approximately in proportion to body weight and if diffusing capacity does indeed reflect alveolar capillary area.

517. CARDIAC OUTPUT IN THE PITUITARY GIANT RAT. A. L. Siegel and J. D. Emerson (intr. by J. M. Bruhn). Dept. of Physiology, Univ. of Alabama Med. Ctr., Birmingham.

The adult Norway rat treated with anterior pituitary growth hormone shows an initial major growth followed by reduced growth and eventual growth cessation (plateauing) irrespective of dosage, purity of the growth hormone preparation or age of the rats at onset of treatment. It has been suggested that cardiovascular changes may be related to this plateauing on growth hormone treatment (Selye. Ann. d'Endocrinol. 13:841, 1952). In the present investigation, cardiac output was measured, by direct Fick, on 6 female pituitary giant rats, plateaued while still on treatment, weighing an average of 634±8.6 gm, compared to 6 adult female controls weighing 275±10.8 gm. Mixed venous samples were obtained from the right atrium by catheterization under light nembutal anesthesia. Arterial samples were taken from the common carotid artery. The treated animals had a mean cardiac output of 144.0±13.4 ml/min (22.7±2.07 ml/min/100 gm body wt). The controls had a mean output of 64.5±4.2 ml/min (23.6±1.6 ml/min/100 gm body wt). Data from one of the treated rats indicates that there is an adequate cardiac reserve. We interpret these data as indicating that there is no meaningful difference in cardiac output of the pituitary ginat and normal control rats ($p = 0.36$).

518. NON-BARBITURATE DEPRESSANTS: EFFECT ON EEG AND CENTRAL POLYSYNAPTIC MECHANISMS. E. B. Sigg,* J. A. Schneider and R. Holland.* Research Dept., Ciba Pharmaceutical Products Inc., Summit, N. J.

Glutethimide, methyprylon and hydroxydione have been studied in their effect on spontaneous brain waves, the thalamo-cortical recruiting response (T.C.R.R.), the brainstem arousal mechanism and on the polysynaptic flexor reflex in the unanesthetized cat with a high spinal transection. The various responses were compared with those obtained under the influence of a barbiturate and chloralosane. At subanesthetic doses (5-10 mg/kg i.v.) all 3 non-barbiturates exhibited a slowing of the brain waves. The threshold for brainstem arousal was elevated and the T.C.R.R. slightly increased. At higher doses (15-30 mg/kg) hydrxoydione often caused organized spindle activity which is known to occur after administration of barbiturates. Although methyprylon also showed distinct grouping of potentials to spindles, irregular slow waves and sharp waves occurred frequently. Glutethimide exhibited a pattern as it is observed under the influence of chloralosane consisting of spike-type discharges and irregular slow waves. At these higher doses the brain stem arousal response was blocked. Parallel to the disintegration of spontaneous spindle activity the T.C.R.R. became increasingly disorganized in the following order: hydroxydione, methyprylon, glutethimide. The polysynaptic flexor reflex was not significantly altered by any of the tested non-barbiturate depressants or chloralosane in doses of 5-30 mg/kg. This is in contrast to the marked depression of the flexor reflex after administration of subanesthetic doses of barbiturates.

519. ALTERATION OF METHIONINE-S^{35} METABOLISM BY URECHOLINE. Melvin Sikov, Robert Weber, Robert Whitrock and James Lofstrom (intr. by C. G. Johnston). Dept. of Radiology and Surgery, Wayne State Univ. School of Medicine, Detroit, Mich.

Healthy mongrel dogs were anesthesized with pentobarbitol and suitable cannulae were placed for complete collection of liver bile and pancreatic juice. Five milligrams Urecholine were administered intravenously. After stabilization of blood pressure with Levofed, L-methionine-S^{35} was injected intravenously. Blood samples were taken at 2 and 60 min and bile and pancreatic juice were collected for 1 hr. Biopsies of liver, pancreas and muscle were taken after 1 hr. Suitable aliquots of the collected fluids and homogenates of the tissues were plated onto planchets, dried and assayed in a gas-flow Geiger counter. Histological sections and autoradiographs were prepared from the tissues. Methionine in pancreatic tissue was greater at 1 hr in Urecholine-treated animals than in controls and hepatic concentration was decreased. Control pancreas and liver specimens showed similar S^{35} concentrations. Urecholine increased the muscle content of radiosulfur although the concentrations never approximated those found in either the pancreas or liver. The concentration of S^{35} in the bile was greater than in the pancreatic juice in control animals. After stimulation with Urecholine S^{35} concentration was decreased in bile and increased in pancreatic juice so that the 2 concentrations approached equality. Urecholine also decreased bile flow and greatly enhanced the secretion of pancreatic juice. The results of this study indicate that Urecholine 1) increased pancreatic tissue uptake of S^{35} and 2) increases both the flow of pancreatic juice and its concentration of S^{35}.

520. EFFECT OF RESPIRATION ON THE SPATIAL VECTORCARDIOGRAM. Ernst Simonson, Otto H. Schmitt and K. Nakagawa.* Lab. of Physiological Hygiene and Dept. of Biophysics, Univ. of

Minnesota, Minneapolis.

In 38 normal men and 60 cardiac patients with various types of pathology the mean spatial QRS and T vectors in mid-respiration, maximum inspiration and maximum exspiration were constructed from conventional ECG leads by means of a mechanical analyzer. In some of these men maximum instantaneous vectors were oscilloscopically recorded using various lead systems: Duchosal; Burger; Wilson-Burch; Frank; 3 bipolar anatomically orthogonal leads (SVEC II); 3 electrically orthogonal leads involving 14 electrode positions (SVEC III). In normal men there was a statistically highly significant forward rotation of the mean T vector (mean + 9.5°) and backward rotation of the QRS vector (mean -8.5°) from midrespiratory position to maximum inspiration and smaller reverse changes in maximum exspiration. The downward movement of the mean QRS vector in maximum inspiration (mean change 16.5°) exceeded that of the T vector (4.5°). There was a significant correlation between mid-respiratory position and respiratory changes in the elevation, but not in the azimuth of the vectors. The discrepancy between respiratory changes of the mean QRS and T vectors were confirmed with oscilloscopic recording of maximum QRS and T vectors which revealed also respiratory changes of spatial loop configuration. The respiratory changes differed greatly in the different lead systems. The respiratory vectorial changes in most patients differed significantly from the normal response. In patients with ventricular bigeminy, the vectorial respiratory changes of the regular and the premature ventricular beats were different. The results cannot be explained by anatomical respiratory changes alone.

521. CONDUCTION OF PERISTALTIC WAVES ACROSS URETERAL ANASTOMOSES. William Sleator, Jr., Harvey Butcher, Jr.* and Werner Schmandt.* Depts. of Physiology and Surgery, Washington Univ. Med. School, St. Louis, Mo.

Action potentials of dog ureteral smooth muscle were recorded with a multiple intralumenal electrode assembly at various intervals after the ureters had been cut across and surgical anastomosis performed. Conduction of peristaltic waves across the anastomosis could not be observed sooner than 21 days postoperatively, but except for one case it occurred at all intervals between 28 and 160 days. There was always conduction delay at the cut ranging from 0.04-1.4 sec. Frequently both arrival and departure waves could be seen on the same electrode located in the anastomosis. Such records showed normal velocities except within the scar and during the delay a prepotential similar to that of cardiac pacemaker cells. Conduction across the cut has important properties in common with conduction in normal ureter: velocity is usually the same in the 2 directions; the velocity of a wave which follows another at an interval less than about 6 sec becomes up to 50% less than normal as the interval decreases; conduction was not significantly influenced by cholinergic or adrenergic drugs or local anesthetics. Near the anastomosis repolarization frequently showed oscillations or multiple small spikes such as replace the plateau in normal ureter at subnormal temperatures. These observations, particularly the long delays, suggest that the mechanism of conduction in the ureter may be the release of some substance by an active cell which diffuses to and activates the next cell.

522. EFFECTS OF SULFHYDRYL BLOCKADE ON AXONAL FUNCTION. H. Millard Smith (intr. by N. R. Brewer). Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

These experiments indicate that blockade of the sulfhydryl (SH) group results in conduction block in frog whole nerve and in the isolated lobster axon, and in reduction of the resting potential in the lobster axon. These effects were produced by the compounds n-ethylmaleimide (NEM), p-chloromercuribenzoic acid (PCMB) and $CuCl_2$. The question of the degree of selectivity of these reagents for the SH group was answered by experiments in which the effects of these reagents on nerve were tested in the presence of small organic molecules with reactive groups similar to those found in protein. It was found that $CuCl_2$ was ineffective in the presence of glycine, glycylglycine, serine, salicylic acid, arginine, cysteine and gluthathione, whereas only glutathione and cysteine prevented the action of PCMB and NEM. In frog nerve, glycylglycine in the presence of both NEM and PCMB and serine and arginine in the case of PCMB did prolong the time to produce conduction block; but this did not occur in lobster nerve. Since only SH containing compounds prevented the action of NEM and PCMB, these reagents were considered selective for SH groups, the status of NEM being the most firmly established. Reversal of effect was obtained in the case of PCMB.

523. COAGULATION MECHANISM IN HEMORRHAGIC SHOCK. James J. Smith, John P. Hartwick and Michael J. Regan (intr. by Robert D. Taylor). Dept. of Physiology, Marquette Univ. School of Medicine, Milwaukee, Wisc.

Unanesthetized dogs previously injected with morphine (1 mg/kg) were subjected to standard hemorrhagic shock using the Wiggers technique. No anticoagulants were used in the cannula systems; blood drawn from a femoral artery was maintained fluid by running it through an ion exchange resin (Dowex 50). After the hemorrhagic period, the withdrawn blood plus equivalent amounts of removed electrolytes were reinfused intravenously; 24 animals were studied and determinations of blood coagulation time, platelet count, hematocrit and prothrombin activity were made before, during and after the hypotensive period. About one half of the animals had a shortening of coagulation time early in the shock period. More characteristic, however, was a prolongation of the coagulation time in the late hypotensive and post-reinfusion period to double or more the control value in about 70% of the animals. Blood platelets and prothrombin activity (Quick) both showed a marked and often parallel decline to levels of 50,000-100,000 platelets/cu mm and 10-20% prothrombin activity respectively. The decreased coagulability of whole blood in the later stages of severe hemorrhagic shock could conceivably be due to a decrease of blood prothrombin complex, or platelets or to heparinemia. (Aided by research grant from Wisconsin Heart Assoc.)

524. RAT PLATELETS IN IRRADIATED, RAT BONE MARROW-INJECTED MICE. L. H. Smith, T. Makinodan and C. C. Congdon (intr. by Alexander Hollaender). Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

It has been established that isologous or homologous bone marrow is an effective recovery agent for lethally x-irradiated mice. Furthermore, the erythropoietic and granulocytic elements of mice given rat bone marrow after x-irradiation are of rat origin. In the present study, immunohematological methods were used to determine whether irradiated mice injected with rat bone marrow had rat or mouse platelets. Species specific rat antimouse and mouse antirat

platelet sera (agglutinins) were prepared. Sprague-Dawley rats and $C_3H \times 101F_1$ mice were used. Lethally x-irradiated (950 r) mice were injected with rat bone marrow and their blood platelets rested against the antisera at periods 7-114 days postirradiation. Rat platelets were detected in mice 7 days after treatment. By the 20th day, all mice tested had rat platelets only. Platelets in mice tested at several periods thereafter were usually of rat origin. A mixture of mouse and rat platelets was found in one mouse 60 days after irradiation; and in one mouse 82 days postirradiation, mouse platelets only were present. The data showed that the appearance rate of rat platelets was greater than that of rat erythrocytes. The 100% rat platelet level was attained in some mice by the 14th day and in all mice by the 20th day, while the 100% rat erythrocyte level is not reached until about the 60th day (T. Makinodan, 1956). The results indicate that rat platelet precursors implant and produce functional platelets in irradiated mice injected with rat bone marrow.

525. HEPATIC $Q_{O_2}^N$ AND MITOCHONDRIAL NUMBER OF COLD-ADAPTED RATS. Robert E. Smith. Physiology Dept., Univ. of California Med. Ctr. and VA Ctr., Los Angeles.

Recent studies (Dennison et al. Fed. Proc., 1955) on the male rat under prolonged exposure to an ambient temperature of 2°C indicate that a marked increase occurs in the endogenous oxygen utilization of liver slice in vitro. These studies have been repeated with individually caged, Long-Evans male rats comparably exposed to 2°C, ambient, but maintained on a high protein diet (Simonsen, White diet) ad lib. Besides metabolic studies made on the liver slices, livers were assayed for mitochondrial number and amount (methods as reported by Smith, Ann. New York Acad. Sci. Jan. 1956) and for total phosphorus and nitrogen content. Contrary to expectation, no increasea appeared either in mitochondrial number or amount, nor were differences obtained in the other chemical and physical assays of the various cell fractions. Respiration measurements, obtained on the liver slices in Warburg flasks with Krebs Ringer-phosphate solution (ph 7.2; bath, 37°C) with and without added substrates (glucose, fumarate-acetate, beta-hydroxybutyrate), likewise failed to show a significant difference in $Q_{O_2}^N$ between the cold-acclimatized and the control series kept at room temperature; despite this, it is possibly worth noting that the means of the former were in general some 20% higher than those of the comparable controls in both the endogenous as well as substrate fortified slices. While the present findings evidently fail to confirm those cited above, it appears possible that this may result from variations either in diet or the strain employed.

526. IN VIVO PERFUSION OF PROXIMAL TUBULE OF NECTURUS KIDNEY. A. K. Solomon, Irwin B. Hanenson,* Joseph C. Shipp,* Erich E. Windhager* and Hans J. Schatzmann.* Biophysical Lab., Harvard Med. School, Boston, Mass.

Studies of two kinds, collections and perfusions, have been carried out on the proximal tubule of the Necturus, using techniques developed by Richards and Walker (Am. J. Physiol. 118:111, 1937). In a series of 9 collections in the distal eighth of the proximal tubule, the collected inulin concentration, as measured by C^{14} inulin, is 1.77±0.65 times the plasma inulin concentration. In perfusion experiments, glucose absorption was measured by disappearance of C^{14} glucose from the tubule. In 6 perfusions at a mean velocity of 0.02 μl/min 65% of the glucose disappeared from the tubule, which agrees with the value of 55% reabsorption obtained by Walker and Hudson (Am. J. Physiol. 118:130, 1937) in 42 perfusions, at a mean velocity of about 0.019 μl/min. In 7 perfusions, water movement was measured chemically using inulin and Na movement was measured with Na^{24}. No net water movement was found in the perfusion experiments, in contradistinction to the results obtained in the collections. Control experiments suggest that this discrepancy should not be ascribed to the presence of mercury in the perfused tubule. On the basis of a two-compartment system the sodium flux out of the tubule has been calculated to be 1280 μμmol/min tubule (equivalent to 1200 μμmol/cm² tubular area, sec).

527. WARFARIN-INDUCED DEFICIENCY OF PTC IN RATS AND ITS CORRECTION WITH VITAMIN K. Theodore H. Spaet and Mona L. Kropatkin.* Dept. of Hematology, Div. of Labs., Montefiore Hosp., New York City.

Sprague-Dawley rats were given Warfarin either by i.p. injection or in drinking water. The daily dose was 0.1-0.3 mg/kg. Blood obtained by cardiac puncture was tested for prothrombin by the Quick method and assayed for plasma thromboplastin component (PTC) with a modified thromboplastin generation test and by the technique of correcting prothrombin consumption in PTC-deficient whole blood. After 4 days of treatment there was no demonstrable prothrombin and PTC was markedly reduced to an estimated 5-15% of control values. Animals on Warfarin for 7 days or more showed a further reduction of PTC. Good correlation was shown between the 2 techniques for PTC estimation. When vitamin K_1 was given by i.p. injection to rats rendered hypoprothrombinemic and PTC-deficient with Warfarin, there was restoration of PTC levels that paralleled the rise in prothrombin. In some animals, normal values of both factors were attained 4 hr after vitamin K_1 injection. The data support the view that PTC is a prothrombin derivative or that both of these clotting factors have a common origin.

528. RELATION OF EOSINOPHIL CELLS TO IMMUNITY. R. S. Speirs. Anatomy Dept., State Univ. of New York, Brooklyn.

The presence of antigenic material in the tissues of an organism not only stimulates a pre-existing network of reticulo-endothelial cells but produces a migration of hematogenous cells into the inflamed area. By studying the quantitative differences in the local cellular responses of actively immunized and passively immunized animals, it is possible to determine which cells are involved as part of the general inflammatory responses to the antigen or the antigen-antibody combination and which ones are present only at the time of antibody production. In experiments involving more than 1000 mice it was noted that a local eosinophilia at the site of injection of a tetanus toxoid antigen was consistently found in actively immunized animals showing secondary antibody responses. However, the eosinophilia was not obtained in non-immunized or passively immunized animals, or in actively immunized mice in which the antibody response had been inhibited by irradiation. Earlier experiments had indicated a similar pattern of eosinophil response following repeated injection of a wide variety of polysaccharide and protein antigens. The response was not obtained following injections of isogenic proteins and similar non-antigenic materials. These experiments indicate that eosinophil cells are involved in immune responses of animals. Their presence at

the site of antigen fixation immediately prior to the appearance of antibody suggests that they play a role in its formation.

529. FACTORS MODIFYING RADIOSTRONTIUM EXCRETION IN MAN. Herta Spencer, Joseph Samachson, Bernard Kabakow and Daniel Laszlo (intr. by Eugene Y. Berger). Div. of Neoplastic Diseases, Montefiore Hosp., New York City.

In studying the calcium and radiostrontium metabolism in patients with metabolic and malignant bone diseases a correlation was noted between renal clearances of calcium and radiostrontium. In order to investigate this relationship further the effect of sudden elevation or of lowering of the ionized plasma calcium upon the renal clearance of calcium and strontium was studied. The elevation of ionized serum calcium was achieved by the slow i.v. infusion of approximately 500 mg Ca^{++} as the gluconate. Marked enhancement of radiostrontium and of calcium clearances was noted. When the ionized plasma calcium was depressed by the infusion of Na-EDTA, a significant decrease of radiostrontium and of ionized calcium excretion was observed. It was previously reported by Charles that the administration of ammonium chloride markedly enhances the urinary calcium and radiostrontium excretion and thereby significantly reduces the radiostrontium body burden. The mechanism of this action was assumed to be due to induced metabolic acidosis causing decreased tubular reabsorption of calcium and strontium, decalcification and decreased deposition of radiostrontium on the bone surfaces. In order to test whether the combination of oral ammonium chloride and of calcium infusions would be more effective than either agent alone, metabolic studies were carried out in 4 phases: control (SR^{85} i.v.), intravenous calcium gluconate, oral NH_4Cl and the combination of oral NH_4Cl and intravenous calcium gluconate. Representative data will be shown to illustrate that the combination of the 2 agents was superior in decreasing the radiostrontium body burden.

530. RELATIONSHIP BETWEEN DEGREE OF COARCTATION STENOSIS AND RESULTING MURMURS PRODUCED IN THE AORTA. Merrill P. Spencer and Adam B. Denison, Jr. Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N. C.

The murmur produced in the aorta below the site of coarctation may be separated from those murmurs present in the collateral channels by direct auscultation of the surgically exposed aorta and by means of a catheter-tip esophageal microphone (barium titanate ceramic). The latter method is most satisfactory, serving well the precise localization of the lesion. When the microphone lies behind the normal aortic isthmus in the dog's esophagus, a short systolic murmur is often heard. During graded constriction with a wire loop the murmur grows in intensity, duration and coarseness until it fills systole and obscures the heart sounds. The internal circumference of the aorta, at this point, is preliminarily estimated to be 6-7 mm. With further stenosis, approaching that usually found in coarctation, the aortic murmur extends into diastole then becomes higher in pitch and softer. Just before complete occlusion (estimated internal circumference 1-2mm) the murmur is continuous with systolic accentuation as in patent ductus. It is, however, of such high pitch and low intensity that it is heard with difficulty at the skin surface. Changes in the murmur with surgical correction follow the pattern of those heard upon gradual release of the experimental loop, or the reverse of the findings upon tightening. The contour of the murmur envelope is similar to the contour of the flow pulse (recorded by means of the surgical probe type square-wave electromagnetic flowmeter) from six aortas senosed by congenital coarctation and from aortas experimentally constricted.

531. INTRACELLULAR RECORDING FROM SMOOTH MUSCLE CELLS OF CAT INTESTINE. Nick Sperelakis* and C. Ladd Prosser. Dept. of Physiology, Univ. of Illinois, Urbana.

Single smooth muscle cells of isolated ganglion-free circular muscle of cat ileum (mounted on a glass rod) were impaled with KCl-filled glass microelectrodes having tip diameters less than 0.5 μ and having resistances of 2-7 megohms. To obtain low input grid currents, an electrometer cathode-follower circuit was used, the output being connected to the DC amplifier of an oscilloscpoe. Membrane potentials were obtained which varied from 20-60 mv, the majority being 35-50 mf. External electrical square-wave stimulation, or stimulation by barium chloride, resulted in action potentials approximately 30-50% of the magnitude of the resting potential (15-30 mv). The responses usually were repetitive, consisting of groups of 2-5 action potentials 0.2-0.4 sec apart; other responses consisted of a single fast spike. Action potential-like fluctuations frequently followed entry of the microelectrode into a cell. Evidence in favor of true intracellular recording is presented by the following: with the microelectrode tip on the surface of the muscle, no action potentials were recorded during spontaneous peristaltic-like contractions or upon electrical stimulation; a quick thrust of the microelectrode (by means of a micromanipulator) into the muscle generally, but not always, resulted in a sharp drop in potential; a quick movement of the electrode while in the muscle resulted in the potential returning sharply to zero; after a prolonged period of time in the heated, moist chamber, many impaled cells responded only to the first few shocks administered.

532. METABOLIC STUDIES WITH HETEROLOGOUS LOW-DENSITY LIPOPROTEINS. Judy A. Spitzer* and John J. Spitzer. Div. of Labs. and Research, New York State Dept. of Health, Albany.

Immunochemical methods, due to their high specificity and sensitivity, lend themselves particularly well to the study of the metabolism of lipoproteins. Human low-density lipoproteins were injected into rabbits either as fresh plasma or as ultracentrifugal preparations from outdated plasma. Both qualitative and quantitative information could be obtained about the fate of lipoproteins by the method of quantitative complement fixation (Meyer et al. J. Immunol. 59: 195, 1948) and by the agar-diffusion technic of Oudin (J. Immunol. 73:322, 1954). Twice-absorbed antiserum against low-density lipoproteins was used (Levine et al. J. Exper. Med. 102:105, 1955). Lipoproteins that were prepared from outdated plasma were removed very quickly from the circulation, showing a halflife of about 1 hr. Fresh, untreated human lipoproteins, on the other hand, had half-lives of many hours and showed individual variations. It is not yet known whether the marked difference in half-lives of the 2 types of lipoprotein preparations is due to the rates of removal or to differences in their metabolism. Similar changes were not found in the half-lives of human albumin or gamma globulins whether these proteins were injected into the rabbit in a purified form or as fresh whole plasma.

533. EFFERENT CEREBELLAR PATHWAYS TO BRAINSTEM. James M. Sprague and William W. Chambers. Dept. of Anatomy and Inst. of Neurological Sciences, Univ. of Pennsylvania, Philadelphia.

Efferent corticonuclear projections of cerebellum in cat are organized in 3 zones: vermal-fastigial, paravermal-interpositus and lateral-dentate (Jansen and Brodal 1940). Unilateral lesions in vermal cortex or fastigial nucleus give bilateral motor deficits of whole body, clearly extrapyramidal in nature. Lesions in paravermal cortex or interposed or dentate nuclei give symptoms in contralateral limbs which are indistinguishable from pyramidal syndrome. Efferent fiber degeneration following unilateral nuclear lesions and use of Nauta-Laidlaw silver technique demonstrates that brainstem projection from fastigius is largely distinct from that of interposed-dentate nuclei, although some overlapping occurs. Each fastigius projects bilaterally (decussation in cerebellum) from rostral thalamus to cervical cord. Projections to medulla and cord are via direct fastigiobulbar tracts, chiefly ipsilateral and indirect, uncinate fascicles, chiefly contralateral. These terminate in all vestibular nuclei, medullary reticular formation and cervical internuncial gray matter. Fibers arising from each uncinate fascicle pass into brachium conjunctivum and ascend brainstem without decussation. This pathway terminates chiefly in midbrain reticular formation and posterior commissure nucleus, intralaminar and midline thalamic nuclei. Interposed and dentate nuclei, however, reach contralateral brainstem via decussation of brachium. The ascending path terminates chiefly in red nucleus, mesencephalic reticular formation, Forels field H , subthalamus, certain specific thalamic nuclei (ventral posteromedial, ventral lateral) and ventral anterior nucleus. The descending ends on trapezoid nuclei and adjacent medial reticular formation. Thus, 2 major efferent systems exist in cerebellum, differentiated by anatomical connections and contributions to motor function. It appears likely that these 2 systems make different contributions to function of thalamus and cerebral cortex. (Supported by PHS #460.)

534. INTRADIENCEPHALIC INJECTIONS OF SALINE SOLUTIONS. Russell D. Squires and Nellie R. Harris (intr. by John R. Brobeck). Depts. of Physiology and Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia.

Intradiencephalic injections of hypertonic NaCl solutions evoked drinking (Andersson. Acta Physiol. Scand. 28:188, 1953) and feeding (Larsson. ibid. 32: Suppl. 115, 27, 1954) in goats. Similar studies were made on 24 cats and 11 rats. In 1 cat and 1 rat drinking responses occurred promptly. In this rat drinking was evoked repeatedly. More often cats exhibited a transient or sustained fixed "starling" appearance sometimes quickly followed by lip-licking, oesophago-pharyngeal movements and circling behavior. Rats exhibited similar activity. Other responses included feeding, tachypnea, panting, defecation, pilo-erection and changes in pupil size. This wide variety of responses including drinking was evoked not only by hypertonic saline injections but also by insertion of the injection cannula and/or injections of isotonic saline. These observations suggest that certain central neurons are stimulated by a variety of mechanical stimuli (Alanis and Mathews. J. Physiol. 117:59P, 1952) including sudden osmotically induced neuron volume changes. The type of response elicited appears to be determined by the individual characteristics of the neuron, e.g. secretion of antidiuretic hormone, or by its efferent connections, e.g. motor responses.

535. BLOOD FLOW RESPONSES IN SKELETAL MUSCLE TO SUDDEN CHANGES IN ARTERIAL PRESSURE. W. N. Stainsby and E. M. Renkin (intr. by J. A. Shannon). Lab. of Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

Responses of the vascular bed of innervated and acutely denervated skeletal muscles in situ to sudden changes in arterial pressure were studied by measuring venous outflow. Sudden, sustained increases or decreases in pressure resulted first in a large transient increase or decrease, respectively, of flow and second, within 1-2 min, a return to a steady flow level nearer the original flow rate. According to previous investigators of these phenomena, the second phase represents an active response of the smooth muscles of the minute vessels to changes in pressure, rather than metabolic regulation of flow. Their conclusions are further supported by our observation that brief changes in pressure followed by return to control levels after as little as 1 sec are sufficient to initiate changes in vascular tone lasting as long as 1 min. The transient change in flow is considered to be entirely passive. Both transient and steady-state pressure flow relations may be modified by vasoconstrictor drugs. The relation of these changes to regulation of the peripheral circulation will be discussed.

536. EFFECTS OF VARIOUS FATS ON CHOLESTEROLEMIA AND ATHEROGENESIS IN CHOLESTEROL-FED COCKERELS. J. Stamler, R. Pick and L. N. Katz. Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.

These experiments were undertaken to assess the effects of various neutral fats—animal versus vegetable, saturated versus unsaturated, low versus high essential fatty acid (EFA)—on cholesterolemia and atherogenesis in chicks fed starter mash supplemented with cholesterol. In one series of experiments, 5% lard and cottonseed oil were compared in birds fed 0.5% cholesterol mash. Levels of cholesterolemia and aorta and coronary atherogenesis were essentially similar in the 2 groups. In the 2nd series, 2 groups of cockerels were fed starter mash + 36.5% egg yolk (cholesterol in feed - 1%, total fat - 13%). One group also received 5% linoleic acid concentrate mixed into the diet. Cholesterolemia and atherogenesis were not significantly different in the 2 groups. In the 3rd and 4th series of experiments, 5%, 20% and 40% corn oil were compared with 5% cottonseed oil added to mash supplemented with 1% and 2% cholesterol respectively. With 5% corn oil, cholesterolemia and aorta atherogenesis were as marked as with cottonseed oil; coronary lesions were less severe. With 20% and 40% corn oil, all values were grossly higher. Under the conditions of these experiments, in chicks fed cholesterol added to commercial mash, saturated animal fat was no more atherogenic than unsaturated high - EFA vegetable fat; high EFA corn oil did not exhibit definitive consistent anti-hypercholesterolemic or anti-atherogenic effects.

537. PERIPHERAL CIRCULATION IN CADAVERS PERFUSED WITH BLOOD AT NECROPSY. Isaac Starr. Dept. of Therapeutic Research, Univ. of Pennsylvania School of Medicine, Philadelphia.

No second systole interrupts the falling curve of diastolic pressure in our cadaver preparations and we have used this curve to define the state of the peripheral circulation. Gomez' parameter $\bar{c}$, the asymptote of the falling pressure curve, estimated mathematically

and regarded as a measure of the "counter pressure" of the peripheral circulation, was calculated from the early portion of our curves, the part available during life. Gomez' parameter θ, the exponential pressure fall rate, regarded as a measure of friction in the peripheral circulation, was also calculated from our curves. Our 43 values are all within the range found by Gomez in patients. The falling pressure curves of our cadavers, exponential in form, level off well above zero pressure. This supports Burton's conception of a "critical closing pressure." This study has produced no evidence that the general dynamic properties of the peripheral circulation of our cadaver preparations were different from those which may be found during life. Extreme reduction of peripheral resistance, found by Guyton et al. was not present in our experiments.

538. EFFECT OF THIAMIN DEFICIENCY ON SECRETION OF ACID BY VAGALLY DENERVATED AND VAGALLY INNERVATED GASTRIC MUCOSA. Patricia A. Stefanik,* Marge K. Lavers,* M. Herbert Smith* and Charles F. Code. Mayo Fndn. and Mayo Clinic, Rochester, Minn.

Vagally denervated, or separated, Heidenhain-type gastric pouches were prepared in one series of dogs, and in another series vagally innervated, or mucosal septal, pouches of the Pavlov type were made. The maximal secretory capacity of both types of pouches in respect to histamine and their response to a standard dose of histamine were determined before, during and after the development of thiamin deficiency. The secretory response of the vagally innervated pouches to insulin hypoglycemia also was tested. The first sign of deficiency of the vitamin was loss of appetite which nearly always developed during the 3rd wk. Weakness and incoordination occurred later. The response of both the vagally innervated and vagally denervated pouches to the standard dose of histamine and the maximal secretory capacity of both types of pouches in response to histamine were unaltered during the deficient state, indicating that thiamine is not concerned with local metabolic processes in the parietal cell. The HCl output of the vagally innervated pouches during nervous stimulation by insulin hypoglycemia was, however, drastically reduced as soon as symptoms of deficiency developed and, as these progressed, the response often was lost. Results indicate that the vagus nerve participates in the neuritis which is characteristic of thiamine deficiency and that this is the cause of alterations in gastric secretion.

539. EFFECTS OF HYALURONIDASE AND HYALURONIDASE INHIBITOR ON FLUID ABSORPTION FROM PERITONEAL CAVITY. Emil Steinberger (intr. by Esther Hardenbergh). Naval Med. Research Inst., Bethesda, Md.

Adult female rats (185±15 gm) were injected i.p. with 15 ml of a 0.9% NaCl solution. Groups of animals were sacrificed at intervals after injection, the fluid from their peritoneal cavities recovered and its volume measured. Three hours after injection approximately 60% of the injected volume was recovered, 20% after 6 hr and 0.0-2% after 12 hr. Intraperitoneal treatment with hyaluronidase for 1 or 2 days prior to injection of NaCl solution significantly increased the rate of absorption. On the other hand, intraperitoneal treatment with hesperidin (a hyaluronidase inhibitor), resulted in a significant decrease in the absorption rate. In animals receiving hesperidin and sacrificed 3 hr after an i.p. injection of NaCl solution 85% of the volume of injected fluid was recovered, 75% was recovered from those sacrificed 6 hr after the injection and 25% after 12 hr. The effect of hesperidin could be entirely abolished by a concomitant intraperitoneal injection of hyaluronidase. In order to establish whether the effect of hesperidin is mediated through the pituitary-adrenal axis, the hesperidin experiments were performed on adrenalectomized and sham-operated animals. The effect of hesperidin was not altered by either procedure.

540. AMMONIA PRODUCTION IN SYMPATHETIC GANGLIA. William J. Stekiel* and Martin G. Larrabee. Biophysics Dept., Johns Hopkins Univ., Baltimore, Md.

Metabolism of endogenous proteins and amino acids was investigated by measuring ammonia output from intact superior cervical ganglia excised from rats. Ammonia was determined by the Conway diffusion method, modified to permit preganglionic electrical stimulation and observation of postganglionic action potentials. With glucose present, ammonia output at rest averaged 2.2±0.5 μM/gm hr (dry weight) during the 2nd hr after excision. Average output decreased with time but the change was not statistically significant during 6 hr. The output was unchanged by supramaximal repetitive stimulation (10/sec). Ammonia output increased progressively following withdrawal of glucose, reaching a maximum (15.2±1.4 μM/gm hr) after about 2 hr, concurrently with failure of the postganglionic response. Restoration of glucose reduced ammonia output to control levels, but the postganglionic response did not recover. Ammonia output was increased (up to 100 μM/gm hr) by addition of glutamate (up to 1 mg/cc) in the presence of glucose but not in its absence. Ammonia was taken up by the tissue when present in a concentration of 60 μM/l. Estimates of oxidation of proteins or amino acids, based on observed ammonia output, accounted for only 25% of the oxygen uptake in the absence of glucose and only 2% of the extra oxygen consumed during activity. It was previously shown that the latter is not accounted for by glucose metabolism. Thus, although a ganglion can apparently metabolize amino acids, either much ammonia is retained by the tissue, or other endogenous materials are also oxidized. (Supported by Grant B 702 from the Natl. Insts. of Health, PHS.)

541. DDT-INDUCED TOXINS IN INSECT BLOOD. James Sternburg, C. S. Chang and C. W. Kearns (intr. by L. E. Chadwick). Dept. of Entomology, Univ. of Illinois, Urbana.

DDT-poisoned roaches contain a substance other than DDT in their blood which is toxic to insects (Science, 116:144, 1952). Susceptible and DDT-resistant house flies injected with blood from DDT-prostrate roaches show symptoms similar to DDT poisoning. The toxin is soluble in water, methanol and ethanol, but insoluble in non-polar solvents. Biological activity of the toxin is destroyed by acetone and alkaline conditions. After dialysis the toxin is stable at neutral or weakly acidic pH. Partially purified toxin diluted about 50-fold from whole blood causes greatly increased spontaneous activity of an isolated roach central nerve cord. Higher concentrations block spontaneous activity. The toxin does not inhibit acetyl cholinesterase in vitro. None of the pharmacological properties of acetylcholine can be observed. It is chemically and chromatographically distinct from acetylcholine, adrenaline, nor-adrenaline and histamine. A substance with the same biological, chromatographic and chemical properties as the DDT-produced toxin can be produced in roaches by repeated mild electrical shocks. A similar toxin has been found

in the blood of DDT-prostrate silkworms, house flies and crayfish. No such biologically active substance has been detected in the blood of the untreated organisms. The toxin may be the result of excessive stimulation of the peripheral nerves and may be normally involved in transmission of nervous impulses. During DDT poisoning the toxin may accumulate in the blood and disrupt the entire nervous system.

542. BIOPHYSICAL ASPECTS OF PROTOPLASMIC STREAMING (MOTION PICTURE). Peter A. Stewart and Babette T. Stewart. (intr. by John Haldi). Dept. of Physiology, Emory Univ., Ga.

Cinephotomicrographs of streaming in slime mold plasmodia will be presented. These films illustrate under low magnification the peculiar sequences of events involved in the periodic reversal of streaming direction. At higher magnifications, they show individual particles moving against, or independently of the main stream. These phenomena are not explained by existing theories of protoplasmic movement. Some results of frame-by-frame kinematic analyses of the films will be discussed.

543. METABOLIC CHANGES IN FAILING HEART-LUNG PREPARATIONS. Joseph G. Strawitz, Woo Choo Lee and Helen Hift (intr. by Quillian R. Murphy). Dept. of Surgery, Dept. of Pharmacology and Cardiopulmonary Research Lab., Univ. of Wisconsin Med. School, Madison.

Previous work has shown that the mitochondria from the failing dog heart in oligemic shock were altered morphologically and phosphorylated inefficiently in Warburg experiments. This report deals with an attempt to demonstrate changes in phosphate metabolism in heart failure induced by several methods and at a level of organization intermediate between the whole animal and the subcellular particles. Starling's heart-lung preparations of dogs are employed, modified to give a closed circulation. Cardiac failure is induced by increasing the right atrial pressure, by administering barbiturates and by hypoxia. Levels of phosphates, glucose, lactic acid and aminoacid-nitrogen are measured in the circulating blood at frequent intervals. The results appear to indicate that in this closed system the phosphate supply is limiting and a dynamic equilibrium is established between the heart and the red blood cells. During normal cardiac function the blood phosphates drop initially until a steady state is reached which may be maintained for periods of 4 hr or more. When failure is induced this state is upset and the phosphate level increases.

544. EFFECT OF AGE ON CALCIUM AND MAGNESIUM CONTENT OF RAT BRAIN. E. Streicher (intr. by J. E. Birren). Section on Aging, Lab. of Psychology, Natl. Inst. of Mental Health, Bethesda, Md.

The brains of Sprague-Dawley rats were halved, frozen-dried and ashed with concentrated nitric acid. The cations were absorbed on a column of Dowex 50, and aliquots of the effluent were assayed colorimetrically for calcium with acid ammonium purpurate and for magnesium by means of the thiazole yellow reaction. The water content of brain tissue obtained from 5-day-old, 3-month-old and 2-year-old rats was approximately 88, 78 and 76% respectively. The magnesium concentration was essentially similar in the 3 groups, i.e. about 13 uEq/gm fresh tissue. The calcium content of young adult rat brain was approximately 4 uEq/gm fresh tissue although higher values were usually observed in material obtained from 2-year-old animals.

545. COMBINED VENTILATORY AND BREATH-HOLDING EVALUATION OF SENSITIVITY TO RESPIRATORY GASES. R. C. Stroud and K. E. Schaefer (intr. by E. S. Mendelson). U.S. Naval Med. Research Lab., New London, Conn.

Previous work indicated the existence of 2 distinct groups of individuals in terms of ventilatory response to CO_2, the one exhibiting high, the other low sensitivity. In order to evaluate further the respiratory characteristics of these 2 groups, a series of parallel ventilatory and breath-holding experiments have been performed. Continuous measurements of alveolar CO_2 (Beckman CO_2 meter) and minute ventilation (Spirogram) were made while each subject breathed 1.5, 3 or 5% CO_2 in O_2, 21% O_2 or 16% O_2 for a 15-min period, at the end of which an alveolar gas sample was obtained for CO_2 and O_2 analysis. Each subject also undertook a series of breath-holding maneuvers after breathing 100, 75, 50, 35, 28, 21, 16 or 10% O_2 for 4 min. End respiratory samples were obtained at the breaking point and analyzed for CO_2 and O_2. On the basis of these data a comparison has been made between the gas tensions occurring at the breaking points and the ventilatory response to similar tensions without breath holding. Such an analysis permits an indirect estimate of the "O_2 sensitivity" of the individual subjects and thus provides an additional basis for comparison on the 2 groups.

546. BLOOD GLUCOSE IN EXPOSURES TO OXYGEN AT HIGH PRESSURES. Larry P. Sullivan* and John W. Bean. Dept. of Physiology, Univ. of Michigan, Ann Arbor.

The elevation of blood sugar in exposures to oxygen at high pressures (OHP) suggests that the protection afforded by hypophysectomy and adrenalectomy against OHP might be explained by an attendant lowering of blood sugar, especially since the medullary factor adrenaline which elevates blood sugar was previously shown to enhance the O_2 toxicity (Fed. Proc. 13, 1954) as did insulin. An attempt was therefore made to determine what effect preliminary elevation of blood sugar would have on the toxicity of OHP. Large mature male Sprague-Dawley rats were selected in pairs of 1 test and 1 control. The test was injected intraperitoneally with 1 cc of a 33% glucose solution, the control with 1 cc physiological saline. Blood sugars of test and control were then determined colorimetrically immediately before (and after) their exposure to O_2 at 90 lb. pressure for from about 20-40 min. They were then decompressed in stages, killed under pentothal anesthesia and lungs removed, grossly examined and weighed. It was found that in the test rats the neuromuscular reactions were more delayed in onset, fewer in number, less severe and the mortality less than in the controls as was also the lung weight. It would appear, therefore, that elevation of glucose preceding exposure provides some protection against OHP and that the initial elevation of glucose induced by OHP is a part of a defense mechanism operating through the hypothalamus and sympathetics which when pushed too far becomes a liability, for reasons other than glucose changes alone, as our work with Drs. Johnson and Smith has indicated. (Supported in part by grant #H-1646 Natl. Insts. of Health.)

547. EFFECT OF CORTISONE, HYDROCORTISONE AND ANDRENOCORTICOTROPHIC HORMONE ON GASTRIC SECRETION. David C. H. Sun and Harry Shay (intr. by Stanley Lorber). Fels Research Inst., Temple Univ. Med. Ctr., Philadelphia, Pa.

Data available from the literature obtained in both dog and man concerning the effect of cortisone and

ACTH on gastric secretion yield conflicting results. In view of the contradictory evidence, we investigated this problem again in the dog. For this report, the effect of a single injection of cortisone, hydrocortisone or ACTH on gastric secretion was studied in 3 dogs, each with a Heidenhain pouch. The effect of these same drugs were then investigated in each dog after resection of the antrum. Every dog served as his own control and each drug study in turn was preceded by a control run. Three complete studies for each drug were done before and after removal of the antrum. A total of 40 experiments were performed on each dog. The results show that these drugs in these experimental preparations unquestionably produced an increase in acid as well as pepsin output. An interesting feature is the relationship of the latent period of action of these drugs to the stimulation of gastric secretion. The possible mechanism of their action on gastric secretion will be discussed.

548. RELATION OF KIDNEY LYMPH TO FLUIDS WHICH FUNCTIONALLY DISTEND THE KIDNEY. H. G. Swann and A. A. Ormsby.* Depts. of Physiology and Biochemistry, Univ. of Texas Med. School, Galveston.

We have postulated that the kidney is naturally distended with blood and interstitial fluid; the latter is thought readily to reenter the vascular system when the distended organ drains after being deprived of its blood pressure. The hypothesis was tested by analyzing kidney lumph, arterial and renal venous blood, and the fluid draining from the kidney after its artery was clamped. Both the distending fluid and lymph had more K, Cl, PO_4 and urea than systemic plasma, but less glucose and only half as much protein. The last quarter of the fluid draining from the distended kidney had a hematocrit of 7%. It had the same amount of Na, Cl and glucose as did lymph, and its protein content, both quantitatively and qualitatively, was virtually the same as that in lymph. But it had 1.7 times more K, 1.6 times more PO_4, and 1.4 times more urea than lymph. The hypothesis is evidently only partly proved by these data.

549. GLUTATHIONE PROTECTION IN X-IRRADIATED EYES. Arnold A. Swanson, Heinrich W. Rose and Jack I. Taube (intr. by H. S. Wigodsky). Depts. of Pathology and Ophthalmology, USAF, School of Aviation Medicine, Randolph AFB, Tex.

A preliminary investigation has been made on clinical and enzymatic changes in rabbit eyes after x-irradiation, both with and without pretreatment with glutathione. Conjuctiva, cornea, anterior chamber, iris, ciliary body and retina showed marked clinical changes, e.g. hemorrhage, edema, hyperemia, on whole head x-irradiation. Pretreatment with glutathione gave significant clinical protection against 800 r and was moderately protective against 2240 and 6000 r. Irradiation with 2240 r and 6000 r resulted in significant decreases in succinic dehydrogenase, cytochrome oxidase and glucose-6-PO_4 dehydrogenase activity for all tissues, when compared with controls. Only the glucose-6-PO_4 dehydrogenase activity of iris and ciliary body appeared not to recover 48 hr after irradiation when glutathione was administered.

550. WATER PERMEATION COEFFICIENTS FOR MEMBRANES CALCULATED FROM OSMOTIC AND TRACER DATA. Robert E. Swanson (intr. by L. F. Nims). Brookhaven Natl. Lab., Upton, N. Y.

In a 2-compartment system separated by a cellophane membrane, the Fick permeation coefficient of tracer water was measured as a function of the total pressure on the system. It was found that the ratio of the tracer coefficient, Q^T, divided by the total pressure was a constant, equal in magnitude to the coefficient measured at 1 atm. of pressure ($Q_1{}^T$). The rate of osmotic flow across the same membrane, using dilute sucrose solutions on one side of the membrane, was also measured. When the results were extrapolated to a one osmolar concentration difference and a correction made for the effect of loss of solute on the measured rate of solvent flow, the resulting osmotic permeation coefficient, Q^O, divided by the sum of the atmospheric plus osmotic pressure, $1 + \pi = 25.4$ (at 25°C), was equal in magnitude to $Q_1{}^T$. The discrepancy between Q^O and Q^T, frequently reported for biological membranes, has been assumed by some authors to be evidence for mass flow occurring in the presence of osmotic pressure gradients. Since the rations, $Q^T/(1 + \Delta P)$ and $Q^O/(1 + \pi)$ were both equal to the same constant $Q_1{}^T$, and further, since the tracer experiments were performed under conditions of no mass flow, it seems unlikely that mass flow does, in fact, account for the apparent discrepancy. (Supported by the Atomic Energy Comm.)

551. BLOOD VOLUME AND OTHER STUDIES IN HAMSTERS X-IRRADIATED WITH UPPER BODY SHIELDED. Marguerite N. Swift and S. Tom Taketa.* U.S. Naval Radiological Defense Lab., San Francisco, Calif.

The longer mean survival time of the hamster (Smith et al. Am. J. Physiol. 183:98, 1955) compared with the rat and mouse (6 vs. 4 days, approximately) following severe intestinal radiation injury prompted a study of certain aspects of the intestinal radiation syndrome in this species. Hamsters were given 2000 r (250 KVP x-rays) with head and thoracic region lead-shielded. The dose used was 100% lethal, with essentially all deaths occurring 6-9 days postirradiation. The following data were obtained from irradiated and control groups sacrificed at intervals up to 7 days after exposure. Circulation blood volume (P^{32} tagged red cell method) was essentially normal on day 3, but declined steadily thereafter (35% loss by day 7). Although some of this decline was due to loss in red cell volume, a greater portion was attributable to plasma volume loss, presumably reflecting fluid loss into the intestinal lumen. The weight of the small intestine contents was moderately increased from day 3 on, owing to an increase in the fluid portion. Whereas the amount of fluid within the cecal lumen was normal on day 3, a 4-fold increase occurred thereafter, corresponding in time with the severe plasma volume loss. Small intestine tissue weight decreased progressively (70% loss by day 7). Weight losses sustained by stomach, cecum and colon were considerably smaller. Blood volume and intestine weight losses progress more slowly in the hamster than in the rat but reach essentially similar terminal values. Differences in gastrointestinal tract contents weights between these species will also be discussed.

552. EFFECT OF CURARIZING DRUGS, COMPOUND 48/80 AND OF PYRIBENZAMINE UPON MOTOR END PLATE. J. Szepsenwol. Dept. of Anatomy, Univ. of Puerto Rico School of Medicine, San Juan.

The effect of histamine releasing agents (tubocurarine, Flaxedil and compound 48/80), of curarizing drugs without any histaminic effect known (Decamethonium and Succinylcholine) and of pyribenzamine hydrochloride upon the motor end-plate of a lizard, Anolis Cristatellus, was studied. Mainly the intercoastal muscles were used. The tissue from control and experimental animals was fixed with Bouin's

field and impregnated with gold chloride. It was then dissociated and mounted in glycerin. It was found that tubo-curarine, compound 48/80 and to some extent Flaxedil produce a swelling of both the axon terminals and their accompanying neurilemma cells. Moreover, during the dissociation of the muscle in glycerin, a relatively large number of motor end-plates detached partially or completely from their muscle fibers. This phenomenon could not be observed in preparations from animals curarized with Decamethonium or Succinylcholine in which the neurilemma cells accompanying the axon terminals appeared slightly more granular than in the controls. In animals receiving pyribenzamine alone or in conjunction with tubo-curarine the motor end-plates appear more or less normal. The conclusion is drawn that the swelling of the motor end-plate produced by tubo-curarine and Flaxedil is due to the release of histamine.

553. MODIFICATION OF PROTECTION AFFORDED BY PRE-EXPOSURE FASTING AGAINST INTESTINAL RADIATION MORTALITY. S. Tom Takata and Marguerite N. Swift (intr. by F. W. Weymouth). U.S. Naval Radiological Defense Lab., San Francisco, Calif.

It was previously reported (Fed. Proc. 15:184, 1956) that 24-hr pre-exposure fasting affords significant protection against 3- to 5-day intestinal deaths in rats given supralethal doses of whole-body x-irradiation. This protective effect has been found to be nullified by feeding fasted animals ad lib. for a 15-min period 1-1/2 hr before, but not immediately before, x-irradiation (900 r, 250 KVP). Five-day mortalities were 70 and 36%, respectively. Intestinal content and tissue weights were determined at the time of exposure in comparably treated groups of rats. It was found that 24-hr fasting caused the mean gut wet weight to drop about 18% below that of fed controls (from 5.6-4.6 gm). Feeding of fasted rats elicited rapid recovery of gut weights which were 5.0 and 5.4 gm for animals fed immediately before and 1-1/2 hours earlier, respectively. The degree of gut weight recovery after re-feeding of fasted rats appeared to be related to the presence of feed substance in the upper gastrointestinal tract, particularly in the intestine, indicating that increase in gut weight, if due to increased local circulation, may be a rapidly responding index of the level of gut activity. These results suggest that the protective effect of pre-exposure fasting may be related to its depressing influence upon intestinal function. It might be postulated from these and the previously reported data that the radiosensitivity of the intestine may be influenced, within limits, by its state of activity at the time of exposure.

554. PARATHYROID INFLUENCE ON CALCIUM ION EQUILIBRATION BETWEEN BONE AND EXTRACELLULAR FLUID. Roy V. Talmage, J. R. Elliott,* R. Davis* and A. C. Enders.* Rice Inst., Houston, Tex.

The influence of parathyroids on the ability of bone to supply calcium to extracellular fluid was studied in nephrectomized rats using a peritoneal lavage technique; 250-gm rats, maintained on calcium-deficient diet, were nephrectomized and parathyroidectomized the day preceding use. Lavage fluid (.84% NaCl and 1.5% glucose) was added and drained through an inguinal catheter. Equilibration levels, rates and effect of this stress on bone were studied by varying the time the fluid remained in animals in repeated washings covering periods up to 12 hr. The following results were obtained: 1) animals with intact parathyroids were able to supply calcium to the extracellular fluid at a higher rate and to higher levels, even when maintained under continuous stress up to 12 hr, than were their parathyroidectomized controls. Significant differences in the calcium supplied were noted even in 10-min washes. 2) Animals without parathyroids were always capable of supplying calcium to extracellular fluid, though at a rate and to a level of approximately 50% of their controls. 3) Areas of bone taken from animals with intact glands showed marked increases in numbers of osteoclasts after as little as 6 hr of stress. Parathyroidectomized animals showed no osteoclastic increase. 4) While parathyroid tissue removed from animals at end of experimental period showed some signs of stress, no marked histochemical changes have yet been observed. 5) Despite the fact that calcium rates were maintained throughout stress period, citric acid levels of wash dropped rapidly after first wash in both intact and parathyroidectomized rats.

555. PEPTIDASE PATTERNS IN BONE MARROW OF RATS TREATED WITH ERYTHROPOIETIC STIMULATING EXTRACTS. Myron Tannenbaum,* Albert S. Gordon, Sam J. Piliero, Paul T. Medici* and Charles D. Siegel.* New York Univ., New York Med. College and St. John's Univ., New York City.

Rabbits were made severely anemic by repeated injections of phenylhydrazine. Acidified boiled filtrates were prepared from the pooled plasmas, livers, spleens, thymuses, lungs, brains, skeletal muscles, bone marrows and packed RBC of these animals. When injected into normal female rats, only the plasma extracts stimulated erythropoiesis as judged by the elevations in peripheral RBC, hematocrit, reticulocyte values and by the increases in the percentages of marrow nucleated RBC. Comparisons were made of the peptidase activity toward diglycine (GG) and triglycine (GGG) in the marrows of the recipient rats. The marrows of only the rats injected with the plasma extracts showed highly significant decreases in GG-ase activity when expressed on a nitrogen or cellular number basis. The activity of this enzyme (cellular number basis) also tended to be somewhat lower in marrows of rats receiving spleen and packed RBC extracts. No definable changes in the marrow GGG-ase activity were noted with any of the extracts employed when the values were expressed on a nitrogen basis. When related to cellular numbers, however, a significant decrease in GGG-ase activity of the marrow was evident in rats receiving spleen extracts. The finding that erythropoietic stimulating activity is associated with a decrease in the GG-ase activity of bone marrow suggests the possible use of this parameter in assessing the relative activities of erythropoietic agents. (Supported in part by grant GS-434 from the Natl. Science Fndn.)

556. ANALYSIS OF IMPULSE TRANSMISSION IN THE SQUID GIANT SYNAPSE. I. Tasaki and S. Hagiwara.* Lab. of Neurophysiology, Natl. Inst. of Neurological Diseases and Blindness, Bethesda, Md. and Marine Biological Lab., Woods Hole, Mass.

The squid giant synapse (Young) was used to analyze the mechanism of synaptic transmission of nerve impulses. A strong hyperpolarization of the pre-synaptic giant axon with an intracellular microelectrode (up to 70 mv) did not bring about measurable electrotonic spread of potential into the post-synaptic axon. "Clamping" the membrane potential of the post-synaptic axon at the level of the resting potential, stimulation of the pre-synaptic axon was found to set up an inward-directed membrane current through the synaptic membrane of the post-synaptic

axon. It was shown that this "synaptic current" observed under the voltage clamp conditions was sufficient to account for production of a synaptic potential. With a hyperfine glass-pipette electrode, it was found that there was a space between the pre- and post-synaptic axons where there was no resting potential. The synaptic potential reversed its sign in this space. The synaptic delay can be accounted for as the time required for the diffusion of some substance across this "synaptic space."

557. EFFECT ON SERUM LIPIDS OF 1300 CALORIES OF DAILY WALKING. Henry Longstreet Taylor, Joseph T. Anderson* and Ancel Keys. Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.

Nine healthy university students ate a diet in which the calories derived from fats (primarily from meats and dairy products) was held constant at 44.5-46.5% of total calories through 4 periods in which no change in weight occurred: 1) 2 wk of sedentary activity with an intake of 3200 Calories; 2) 10 days of increasing exercise and calorie intake; 3) 3 wk of 1300 Calories of daily walking (2 hr) on the motor-driven treadmill (3.5 mph at 10%) on an intake of 4250 Calories; 4) 1 wk of sedentary activity on an intake of 3100 Calories. At the ends of periods 1, 3 and 4 concentrations in the serum were estimated for total cholesterol, cholesterol in the alpha and beta lipoprotein fractions, total fat, non-esterified fatty acids and lipid phosphorus. Mean serum total cholesterol averaged 158.5, 160 and 162 mg/100 ml at the ends of the periods 1, 3 and 4, respectively. Significant changes were similarly absent for the other serum constituents in spite of an average increase of 64 gm of animal fat/day during period 3. It is concluded that, in calorie balance, the serum cholesterol concentration is independent of the calorie intake, absolute fat intake and physical activity level when the kind of fat and the percentage of fat calories in the diet are constant.

558. EFFECT OF FOOD INTAKE ON LIPOGENESIS BY LIVER SLICES IN VITRO. Jay Tepperman, Helen M. Tepperman, Margaret Wilson* and Janet M. DeWitt.* Dept. of Pharmacology, State Univ. of New York, Upstate Med. Ctr., Syracuse.

Studies on the glucose R. Q. of obese rats, periodically over-fed rats and of liver slices of the latter (Yale J. Biol. & Med., 15:854, 1943) led to the hypothesis that hyperphagia is associated with increased rates of lipogenesis both in the liver and in some peripheral tissue. In the current study measurements were made of lipogenesis rates from acetate-1-C^{14} and glucose-U-C^{14} by surviving liver slices of gold-thioglucose obese mice, periodically over-fed rats and rats starved for 48 hr and fed a high carbohydrate diet for 24 hr. In all of these circumstances enhanced rates of incorporation of the substrate label into fat were observed under conditions to be described. A high degree of correlation between initial glycogen level and lipogenesis was found. Possible mechanisms involved in the regulation of the widely fluctuating lipogenesis rates seen in these experiments will be discussed in the light of recent studies on fatty acid synthesis.

559. TISSUE CO_2 CONCENTRATIONS IN THE HYPERCAPNIC RAT. Alan M. Thompson and E. B. Brown, Jr. (intr. by Victor Lorber). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

As part of an investigation of the chemical changes in tissues resulting from hypercapnia, post-mortem tissue CO_2 concentrations have been measured in the rat following exposure of the live rat to high concentrations of CO_2. The tissue is rapidly frozen to liquid nitrogen, ground and transferred to 1.0 N NaOH. It is then digested at 60°C. and the CO_2 determined by a modified Van Slyke technique. Control values in the skeletal muscle averaged 13.2 mM CO_2/kg wet tissue; following exposure to 30% CO_2-70% O_2 for 1 hr the skeletal muscle concentration averaged 29.2 One purpose of the investigation was to find how tissue concentrations change after the circulatory stasis of death. Two hours after decapitation the hind limb skeletal muscle concentrations were 14.2 and 27.5 mM/kg in groups breathing air and 30% CO_2 respectively. Since the standard error is 0.4-0.7 in these averages there appears to have been no significant change in tissue CO_2 concentration during the first 2 hr post mortem under these conditions. The fraction of tissue CO_2 precipitable by barium ion has been found to vary greatly and is probably not as applicable to the in vivo forms of CO_2 as is assumed by some workers. The results for other tissues and under other experimental conditions will be presented and discussed in terms of the distribution of CO_2 capacity in the body and the chemical forms of tissue CO_2.

560. RENAL VASCULAR SHUNTS AND AUTOREGULATION OF BLOOD FLOW AND FILTRATION RATE. David D. Thompson, Frederic Kavaler* and Rodolfo Lozano.* Dept. of Physiology, Cornell Med. College, New York City.

Pappenheimer and Kinter have recently proposed that the erythrocytes and plasma entering the kidney are separated to some degree and traverse the kidney by different circulatory paths. This has been based in part on their finding that renal PAH extraction ratios fall in acutely produced anemia. It has been further postulated by these authors that separation of erythrocytes from plasma plays a role in autoregulation of renal blood flow (RBF) and filtration rate (GFR). Previous studies in this laboratory have demonstrated autoregulation of RBF and GFR, renal arterial pressure being varied by inflation of a balloon catheter. In the experiments to be reported PAH extraction ratios were also measured by the additional technique of renal vein catheterization. Results, in 3 dogs, of varying renal artery pressure (by aortic balloon and by hemorrhagic shock) confirm those of others that renal PAH extraction remains constant over a wide range of arterial perfusion pressures (60-140 mm Hg). In two experiments in dogs our dindings are in accord with those of Pappenheimer that renal PAH extraction falls in acute anemia (to 86% of initial value at hematocrit of 10, to 72% at hematocrit of 5). The postulated role of red cells in regulation of RBF is compatible with our findings of an increase in RBF of 28% of its initial value at a hematocrit of 10 and of 60% at a hematocrit of 5. GFR, however, showed no impressive change in acute anemia, the measured increments being 8 and 12% respectively.

561. STRUCTURAL CHANGES IN AXONS EXPOSED TO ENZYMES WHILE STILL FUNCTIONING. Julian M. Tobias. Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

The lobster cord giant axon exposed to phospholipase A or C exhibits rising threshold and decreasing conduction velocity terminating in inexcitability and depolarization. Phospholipase C treatment in addition causes the axon to appear as if sprinkled with refractile particles (30X). Treated with trypsin, chymotrypsin, or both, the axon can become extremely soft and sticky and penetrable by a micro electrode without any dimpling distortion or movement, though electrical

activity continues unchanged. These experiments have been repeated but now with electronmicroscopy in addition (osmium fixation). The following will be discussed and illustrated: 1) immediately fixed control axons show complex structuring with organelles, tentatively named mitochondria, in the axoplasm just inside the surface structures. Surrounding the axoplasm are usually multiple, osmiophilic laminae made up of several layers each and encompassing nuclei (see also Geren and Schmitt, Symp. on Fine Structure of Cells, Interscience, 1955). 2) Prolongedly soaked control axons show surface structure swelling with blurring of outlines, though function remains intact. 3) Protease treatment leads usually to granular destruction of all the periaxoplasmic laminae though function is intact at time of fixation. 4) Phospholipase C treatment, resulting in functional failure, leads to distortion but not destruction of the laminae and the appearance of densely osmiophilic particles scattered among the laminae. It is tentatively concluded that at least certain skeletal protein moieties in axonal surface structure are unnecessary for impulse conduction, but the integrity of certain phospholipids is essential. One must remember that this technique reveals the resultant of experimental treatment plus exposure to the entire fixation-imbedding procedure. Changes produced directly by the enzymes may either be amplified or distorted or both by the subsequent treatment.

563. UNITARY RESPONSES OF "BETZ" CELLS TO ANTIDROMIC AND ORTHODROMIC EXCITATION. Arnold L. Towe and Harry D. Patton (intr. by T. C. Ruch). Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.

In 16 cats under chloralose-decamethonium anesthesia, unitary activity evoked by contralateral footpad stimulation was recorded from 140 cortical units in somatosensory area I using methods described by Amassian. Surface responses were simultaneously recorded. Units (Betz cells) projecting into pyramids were identified by their ability to follow antidromic stimulation of bulbar pyramid at repetition rates of 100/sec or greater. This criterion excludes units orthodromically fired by stimulus spread to medial lemniscus; these fail at 10-20/sec. Forty-five Betz cells were observed at depths ranging from 523-1830 μ with almost uniform distribution. Antidromic spikes showed only minor differences from orthodromically evoked spikes. Threshold response to antidromic stimulation was invariably a single spike; no evidence of re-excitation via recurrent collaterals was found. Moreover, combined antidromic and orthodromic excitation failed to reveal depression of responsiveness beyond that expected from refractoriness (1-2 msec) following antidromic invasion; inhibition via recurrent collaterals was not confirmed. Antidromic conduction time ranged from .67-7.7 msec; if a conduction distance of 43 mm is assumed, computed velocities range from 64-5.6 m/sec. Orthodromic excitation commonly evoked repetitive firing in Betz cells with a number of spikes per discharge significantly greater than that of firing in non-Betz cells. Some units (Betz and non-Betz) were fired by both ipsilateral and contralateral forepaw volleys, the latencies of responses to ipsilateral excitation being longer and more variable. (Aided by grant B-895, PHS.)

563. METABOLISM OF C^{14}-4-CORTISOL IN THE DOG HEART-LUNG PREPARATION. Randall H. Travis, Kathryn Ballard, Neil Solomon and John Bukovnik (intr. by Reginald Shipley). Dept. of Physiology, Western Reserve Univ., Cleveland, Ohio.

After stabilization of mean blood pressure at approximately 100 mm Hg, about 150 μg of C^{14}-4-cortisol were added to the perfusion reservoir. When, as a result of progressive pulmonary edema, pressure could no longer be maintained by adjustment of peripheral resistance and perfusion pressure, affluent and effluent tubes were clamped, the heart and lungs removed from the thorax and the blood drained from the system. Blood was hemolysed and tissues were homogenized. Specimens were extracted with ethanol. Chloroform was added to the extracts resulting in separation into aqueous and chloroform-ethanol layers. Fats were removed from the dried chloroform-ethanol fraction by hexane-ethanol partitioning. Final ethanol extracts were chromatographed in a toluene-propylene glycol system. Strips were eluted in 1-in. sections. About 65% of the radioactivity could be accounted for. Only negligible amounts were found in the hexane and aqueous fractions. Practically all of the radioactivity recovered from blood and from lungs was in a fraction having the chromatographic mobility of cortisol and reacting with phenylhydrazine, absorbing maximally at 410 mμ. Approximately 10% of the radioactivity recovered from the heart was in a compound more polar than cortisol and non-reactive with phenylhydrazine. It is concluded that the dog heart-lung preparation metabolizes cortisol at a very low rate. Virtual absence of radioactivity from the aqueous fraction is evidence against conjugate formation. The only metabolite detected is probably reduced at C-20.

564. INFLUENCE OF INSPIRED O_2 TENSION ON PULMONARY DIFFUSING CAPACITY FOR CO. G. M. Turino,* M. Brandfonbrener,* R. M. Goldring* and A. P. Fishman. Dept. of Medicine, Columbia Univ., New York City.

It is recognized that the pulmonary D_{CO} may reflect the rate of combination of CO and Hb as well as the inherent characteristics of the diffusing surface of the lung. In vitro observations suggest that this rate of combination, and the pulmonary capillary P_{CO}, may be altered by varying the pulmonary capillary P_{O_2}. In the present study, using a steady state method, the effect of change in pulmonary capillary P_{CO} on V_{CO} and D_{CO} was evaluated by successively exposing 9 normal subjects and 3 patients with diffuse pulmonary fibrosis to the same inspired CO concentration (0.1%) in different mixtures of O_2 and N_2 (21, 50 and 100% O_2). Each experiment entailed an initial, 15-min period of breathing one O_2 mixture, followde by a 6-min exposure to the same $F_{I_{O_2}}$ plus 0.1% CO. V_{CO} was calculated from analyses of $F_{I_{CO}}$ and $F_{E_{CO}}$ by an infra-red analyzer. $P_{A_{CO}}$ was calculated from the Bohr dead space relationship assuming $V_{D_{CO_2}} = V_{D_{CO}}$. Pa_{CO_2} was assumed equal to PA_{CO_2}. Pa_{CO} was measured by the method of Allen and Root. In all subjects the low $F_{I_{O_2}}$ increased D_{CO} (average of 63% with a range of 23-120%), whereas, the high $F_{I_{O_2}}$ decreased D_{CO} (average 37% with a range of 31-47%). V_{CO} varied directly with D_{CO}. Increases in V_{CO} while breathing 100% O_2 were greater than predicted from increases in V_E. These results indicate that, in the steady state method, variations in $F_{I_{O_2}}$ induce inverse changes in V_{CO} and D_{CO}; these effects are probably

related to the influence of pulmonary capillary P_{O_2} on the reaction rate of CO and Hb.

565. EFFECTS OF .08 AND .8 ATMOSPHERES OF INSPIRED pO_2 UPON CEREBRAL HEMODYNAMICS AT A "CONSTANT" ALVEOLAR pCO_2 OF 43 mm Hg. J. Turner, C. J. Lambertsen, S. G. Owen, H. Wendel and H. Chiodi (intr. by James E. Eckenhoff). Lab. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia.

To eliminate the powerful effects upon cerebral vessels of small alterations in arterial pCO_2, the present study of cerebral hemodynamics during inhalation of 8, 21 and 80% O_2 at one atm. was carried out at an alveolar pCO_2 adjusted in 6 normal subjects to a mean of 43 mm Hg in each of the experimental conditions. In the absence of significant alterations of alveolar or arterial pCO_2, 80% O_2 produced no change in brain circulation or cerebral vascular resistance from control levels obtained during 21% O_2 breathing. This suggests that the cerebral vasoconstriction normally associated with O_2 inhalation is predominantly an indirect effect, secondary to arterial hypocapnea. Inhalation of 8% O_2, resulting in arterial and cerebral venous pO_2 of 39 and 28 mm Hg, respectively, decreased cerebral vascular resistance 29%, increased brain blood flow 36% and left cerebral oxygen consumption unaltered. The observed degree of cerebral vasodilitation represents the action of low pO_2, unmodified by antagonistic effects of the hypocapnea normally associated with hypoxia.

566. OXALOACETATE AS A REGULATOR OF METABOLISM WITHIN THE KREBS CYCLE. David B. Tyler. Univ. of Puerto Rico, School of Medicine, San Juan.

Evidence indicates that reactions within the Krebs cycle are a major energy source for the cell. Rate regulation within this cycle is, therefore, important. Although many substances have the ability to inhibit the activity of certain components of this system, such capacity alone does not qualify for a major governing role. Certain other necessary characteristics are also required: 1) the substance should be normally available at the site of an enzyme which controls a key reaction in this sequence of metabolic events; 2) it should have a high affinity for the enzyme, reversibly inhibiting activity in minute amounts; 3) its production, utilization and capacity to inhibit should be very sensitive to slight alterations in the environment; 4) naturally-occurring changes in concentration (or availability) should be reflected by corresponding changes in activity. Experiments show that oxaloacetate, a product of succinate oxidation, possesses these qualifications and that it has profound regulatory effects on succinoxidase activity, a "main-line" enzyme system. It has an affinity for succinic dehydrogenase 1000 times greater than the normal substrate; as little as 10^{-5} M produces 50% inhibition. Under physiological conditions, non-inhibitory complexes with ATP and Mg^{++} are rapidly formed. These are readily dissociated by Ca^{++}, releasing oxaloacetate for inhibition. Its utilization, production and ability to inhibit are sensitive not only to the Ca^{++}/Mg^{++} ratio but also to the concentration of other Krebs cycle intermediates and to structural alterations. Finally, under a variety of experimental conditions, the rate of succinoxidase activity is inversely related to the oxaloacetate concentration. (Supported by the Life Insurance Med. Research Fund.)

567. CHANGES IN PROTEIN CONFIGURATION, PROTEASE ACTIVITY AND SH GROUPS IN STIMULATED RAT BRAIN. G. Ungar, D. V. Romano* and E. Aschheim.* Dept. of Pharmacology, U.S. Vitamin Corp., New York City.

Extracts of rat cortex, removed after freezing in situ, were examined for ionized side-groups of proteins (by means of a spectrophotometric method), hydrolysis of the synthetic substrate N-acetyl-L-tryptophan ethyl ester, -SH and S-S groups (by amperometric titration) and total and acid-soluble proteins. After electrical stimulation of the central end of both sciatic nerves for periods varying from 30 sec to 20 min the cortex showed significant increases in protease activity and in ionized side-groups, especially in directly titratable -SH groups. The changes were readily reversible after short stimulation, more slowly after prolonged application of stimulus. Changes were also observed under the influence of anesthetics and other drugs with excitatory or inhibitory action on the central nervous system. A tentative interpretation of these observations is that, under the influence of stimuli, the proteins of excitable structures undergo reversible denaturation with activation of protease. The resulting molecular rearrangement may be responsible for the ionic shift and ultimately for the electrical phenomena of excitation.

568. 5-HYDROXYINDOLEACETIC ACID EXCRETION IN MAN. A. J. Valcourt (intr. by H. E. Himwich). Galesburg State Research Hosp., Galesburg, Ill.

The excretion of 5-hydroxyindoleacetic acid (5-HIAA) in urine was studied before, during and after the daily oral administration of 4 mg of reserpine to mental patients. Two types of middle-aged patients were used, mental defectives and schizophrenics. Twenty-four hour urine collections were made and their completeness was checked by determining the creatinine content. 5-HIAA was extracted and assayed by the procedure of Udenfriend (Udenfriend, Titus and Weissbach, J. Biol. Chem. 216:499, 1955). It was expressed as mg of 5-HIAA/24 hr, and as mg of 5-HIAA/gm of creatinine. Calculated either way, there was an average increase of more than 25% in the excretion of 5-HIAA on the first day of reserpine medication. This was followed by a drop to premedication levels or less. These levels remained approximately the same after cessation of medication. At least 6 wk after the first reserpine study, the same group of patients was given a second course of reserpine. The drug again produced a marked rise in the excretion of 5-HIAA on the first day of medication. The excretion pattern of 5-HIAA in these experiments was approximately the same in both mental defectives and schizophrenics.

569. EXCHANGE OF Na^{24} WITH ETHER EXTRACTABLE Na OF MUSCLE AND BRAIN. John C. Vanatta, Allen F. Reid* and Ann Griffin.* Depts. of Physiology and Biophysics, Univ. of Texas Southwestern Med. School, Dallas.

Purified ether extract of brain was dissolved in 70% alcohol, buffered to pH 7.40±.02 and incubated at 37°C. High specific activity Na^{24} was then added. At 5 min, 1 hr and 24 hr aliquots were removed, dried and the specific activities of the sodium in the petroleum ether-soluble and the water-soluble fractions were determined. There was less than 3% exchange of the sodium in the lipid fraction at 24 hr. Experiments were similarly carried out using 9 rats loaded with Na^{24} by stomach tube. Three rats were sacrificed

at each of the following intervals: 5 min, 1 hr and 13 hr. Brain and muscle samples were taken and specific activities were determined on the plasma and on the purified ether extracts. The ratio of specific activities of brain to the corresponding plasma at the various times averaged 6.4%, 20.7% and 145.3%. A similar increase in the corresponding ratios for muscle to plasma was noted with averages of 13.9%, 29.8% and 92.0%. Although no evidence is available as to the site of the lipid substance(s) in the tissues, the authors suggest that it best fits into a theory of membrane structure and is concerned with the sodium pump mechanism. (Supported in part by the American Heart Assoc., and in part by research grant H-1574-C2 from the Natl. Heart Inst.)

570. POST-MORTEM BRAIN LACTIC ACID AS INFLUENCED BY HYPOXIA AND/OR HYPOCAPNIA. Donald D. Van Fossan,* Clyde Biddulph and Dominic Criscuola.* Dept. of Physiology-Biophysics, School of Aviation Medicine, USAF, Randolph AFB, Texas.

Elevated brain lactic acid concentration post mortem is a potentially suitable criterion for establishing immediate ante-mortem altitude exposure. It was desired to determine whether hypoxia without simultaneous hyperventilation or hyperventilation alone would elevate the post-mortem brain lactic acid. Comparison was made of the 3-hr post-mortem brain lactic acid concentration of the following 5 groups of 8 dogs each: Control, hyperventilated, hyperventilated plus hypoxic and two hypoxic groups. All animals were nembutalized and mechanically ventilated. Significant elevation of the post-mortem brain lactic acid was noted only when hyperventilation and hypoxia were combined. Brain samples from 88 human aircraft crash victims were analyzed for lactic acid. Eighteen samples were higher than the control values for mice, rats, rabbits and dogs and comparable to the values for hyperventilated plus hypoxic dogs. Although the human data may not be compared unequivocally with animal data, the consistency of control values from various species suggests that those humans whose lactic acid values were above 200 mg % were hypoxic and hyperventilating.

571. VALIDITY OF USING I^{131} AS A LABEL FOR DIETARY AND INTRAVENOUS FAT. E. Van Handel* and D. B. Zilversmit. Div. of Physiology, Univ. of Tennessee, Memphis.

I^{131} labeled triolein has been used as an indicator of fat absorption in patients and experimental animals. We have investigated whether the I^{131} label follows the fate of the carrier fat after intravenous and oral administration to dogs. When I^{131} triolein was mixed with cottonseed oil and injected as an emulsion, the I^{131} triolein disappeared at the same rate as the carrier fat. To investigate the effect of the gastrointestinal tract on the stability of I^{131} fat, alimentary lipemia was produced by feeding cottonseed oil mixed with I^{131} triolein. The appearance and disappearance of radioactive triglycerides in plasma agreed closely with that of the chemically determined triglycerides. Determination of the total radioactivity of blood leads to erroneous interpretations due to the early appearance of non-lipide radioactivity from breakdown products. The following methods were used to separate lipide from non-lipide fractions: 1) precipitation of total lipides with trichloroacetic acid in the presence of KI (according to Turner, unpublished), 2) dialysis of plasma, 3) extraction with chloroform-methanol and subsequent washing with aqueous KI solutions, 4) absorption on Doucil and extraction of cholesterol esters and triglycerides with chloroform (Forbes, J. Lab. Clin. Med. 16:520, 1930). Chemical triglycerides were determined from esterified glycerol on this extract by the method of Van Handel and Zilversmit (South. Chemist 16:147, 1956). (Supported by USPHS Grant # H-2181.)

572. ASPHYXIAL VOLUME CHANGES OF CORTICAL CELLS AND FIBERS. A. van Harreveld. California Inst. of Technology, Pasadena.

The sudden and marked drop in cortical conductivity observed about 3 min after arrest of the blood circulation has been taken as an indication that at the moment ions accompanied by water move from the intercellular spaces into the cells and fibers of the cortex (Am. J. Physiol. 187:180, 1956). The conductivity drop is so large that the postulated swelling of cells and fibers might be detectable. The cortex of rabbits before and after the sudden resistance change was quickly frozen with alcohol cooled to its freezing point. Then the brain was stored in alcohol at -20°C for 1 wk. The alcohol dissolves the ice in the cortex and the slowly penetrating alcohol can be expected to fix the tissue more or less in the state in which it was frozen. From each brain pieces of cortex were removed from identical regions. They were embedded, sectioned and stained either with silver for the measurement of fibers, or with gallocyanin for the measurement of cells. From each cortex 200 cells and 100 opical dendrites were measured. The cells after the resistance change were about 10% larger in diameter than before the conductivity drop; the apical dendrites had increased 25-30%. The differences in diameter signify an approximately 30% volume increase of the cells and a 50-70% volume increase of the apical dendrites. These measurements support the thesis that the sudden drop in cortical conductivity due to asphyxiation is caused by a movement of ions accompanied by water from the intercellular compartment into the cellular elements of the cortex.

573. EFFECT OF COLD ON THIAMINE-DEFICIENT RATS. David A. Vaughan and Lucile N. Vaughan (intr. by A. P. Gagge). Arctic Aeromedical Lab., APO 731, Seattle, Wash.

Fifty young adult rats (220-270 gm) were divided into 2 groups. Twenty-five were subjected to moderately severe cold (5°C); 25 were maintained at 25-28°C. Each group was divided into 5 sub-groups, which received a thiamine-deficient diet for 18 days, supplemented with the following levels of thiamine: 0.3 mcg/gm diet, 0.4 mcg/gm, 0.5 mcg/gm, 0.6 mcg/gm and 2.0 μg/gm. Respective weight changes at these levels were: -56 to -84 gm, -27 to -75 gm, -33 to -75 gm, -13 to -26 gm and +17 to +42 gm for the cold rats and -81 to -99 gm, -50 to -110 gm, -37 to -59 gm, -7 to -56 gm and +48 to +53 gm for the warm rats. Corresponding mean voluntary food intakes were 14.1, 16.6, 16.9, 19.5 and 24.2 gm/day for the cold rats and 5.7, 5.4, 7.2, 8.4 and 16.7 gm/day for the warm rats. A statistical analysis indicated that there was no significant difference in weight response between the warm and cold rats at deficient levels of thiamine intake. The relation of weight changes to food intakes suggested that the cold rats used an approximately constant increment of food for their extra energy requirements in the cold. This increment indicates that needs for thiamine in the cold are governed by increased caloric requirements.

574. EFFECT OF METHOCARBAMOL (NEURAXIN) ON HUMAN SKELETAL MUSCLE HYPERACTIVITY. Francis A. Vazuka,* Norma Hajek-Nichols and Jack

Freund.* McGuire VA Hosp. and Med. College of Virginia, Richmond.

3-o-Methoxyphenoxy-2-hydroxypropyl-1-carbamate, called Methocarbamol (Neuraxin), acts as an interneuronal blocking agent as evidenced by its protection of animals against lethal doses of strychnine. The effects on skeletal muscle hyperactivity in 12 individuals with various neurological disorders were observed and compared with electromyographic and myometric studies. Four of the 7 individuals with multiple sclerosis exhibited marked clonus, spasticity and extensor spasms. There was a reduction or disappearance of these manifestations following both intravenous and oral administration of Methocarbamol. A general reduction in hyperreactivity resulted in the other three. These observations were confirmed electromyographically. The clonus which was present in both individuals with cord injuries was controlled by the Methocarbamol regimen. The subject with syringomyelia exhibited only hyperreactivity which was decreased while maintained on this drug. The extreme severity of the spasms seen in the individual with cervical myelopathy was reduced. The reduction in neuromuscular hyperreactivity in all cases was apparent within minutes after intravenous administration and the effect persisted from 4-24 hr following a single dose, depending on the severity of the neuromuscular symptoms. There were no toxic manifestations following either mode of drug administration. Methocarbamol did not produce any increased muscle weakness. Prostigmine did not alter the effect produced by Methocarbamol.

575. EXCRETION OF MALIC ACID IN RELATION TO TRICARBOXYLIC ACID CYCLE IN KIDNEY. P. Vishwakarma (intr. by Gustav Eckstein). Dept. of Physiology, College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio.

The mechanism of excretion of malic acid and the effect of the succinic dehydrogenase inhibitor, malonic acid, were studied in the dog during infusion of sodium salts of citric, succinic, fumaric and L-malic acids. Plasma malate was elevated by each acid except citric. In each case the mechanism of malate excretion differed. With L-malate infusion, maximum malate reabsorption was 15-25% of the filtered load. With fumarate there was also increased malate filtration and reabsorption but the maximum observed reabsorptive rate was increased to 50% of the filtered load. In both these cases malonate was without effect on malate reabsorption. With succinate infusion the picture was entirely different. Filtered malate was increased but here there was in addition a marked tubular secretion of malate. This secretion was inhibited by malonate converting the mechanism to one of filtration and met reabsorption (confirmation of Craig and Miller, 1953). Infusion of citrate, while causing no change in the endogenous plasma level of malate, did cause a marked increase in the excretion of this acid, apparently by inhibiting its tubular reabsorption. From these findings it appears that the renal excretion of malic acid involves variously (and depending on the precursor being administered) glomerular filtration, tubular reabsorption, tubular secretion and/or synthesis and in all likelihood simultaneous bidirectional tubular flux of malate. The relation of these findings to the operation of the tricarboxylic acid cycle in the excretion of cycle intermediates is discussed.

576. RATE OF RECOVERY FROM GAMMA-RADIATION INJURY AS A FUNCTION OF AMOUNT OF INJURY. Howard H. Vogel, Jr., John W. Clark and Donn L. Jordan (intr. by Douglas E. Smith). Div. of Biological and Med. Research, Argonne Natl. Lab., Lemont, Ill.

An experiment was designed to test the hypothesis that the rate or rates of recovery from radiation injury are a function of the amount of injury present. The general plan was to give varying doses of Co^{60} γ-rays (234-884 r) to 5 groups of 84 female CF no. 1 mice (20-25 gm). These first doses corresponded to 25, 50, 65, 83 and 95% of the LD 50/30, single-exposure to Co^{60} (930 r). After a period of 5 days, a 2nd dose of -rays was given to the mice. The amount of additional radiation necessary to kill a portion of the mice in 30 days was calculated, assuming an exponential type of recovery and a recovery rate constant of approximately 17%/day. If one knows in each case the single dose which would result in the lethality observed after the 2 exposures, it is necessary only to subtract the 2nd dose to obtain a measure, in terms of roentgens, of the residual injury from the 1st dose. When this injury is plotted as a function of the 1st dose, it is evident that the size of the residual dose, in r, depends on the amount of the 1st dose. For example, 5 days after 234 r, only 94 r (40%) is residual, while 89% or 783 r is residual after a 1st dose of 884 r. The relation between the daily recovery constant and the injury (expressed as a fraction of the LD 50/30, single-exposure) is described. These data indicate a progressive retardation of the recovery process as doses are increased above the level of half the single-exposure LD 50. Other experiments on radiation recovery will be discussed in relation to these results. (Performed under auspices of the U.S. Atomic Energy Comm.)

577. POSTURAL REACTIONS ELICITED BY BRAIN STIMULATION THROUGH IMPLANTED ELECTRODES. Irving H. Wagman, C. A. Papatheodorou* and Morris B. Bender. Dept. of Neurology, Mount Sinai Hosp., New York City.

Conjugate eye movements may be elicited upon electrical stimulation from wide areas of cerebrum and braim stem of cervically transected monkeys. Since eye movements may form an integral part of wider patterned movements, the present study was undertaken. To date 96 points in brain stem and cerebrum have been stimulated via chronically implanted electrodes in 5 monkeys and 9 cats. Patterned head and eye and/or body and limb movements may be elicited upon stimulation of many brain areas. Responses obatined from cerebrum are simpler than those obtained from brain stem since they usually involve head and eyes primarily and are always contralateral to the side stimulated. In the brain stem the responses are varied. Body movements may or may not be accompanied by head and eye movements, and vice versa. Head and eye movement in either direction may be in association with either contralateral or ipsilateral body movement. Other reactions obtained were appropriate limb reactions associated with head and body movements and complex body and head responses resembling classical righting reactions. Thus far inhibition of movement has not been obtained from stimulation of brain stem. Nystagmus was elicited from all parts of the brain, but was not a constant response from any one point. (Aided in part by USPHS Grant B-294.)

578. INFLUENCE OF STREPTOCOCCUS TOXIN ON THE COURSE OF CANINE EXPERIMENTAL NEPHROSIS. Khalil G. Wakim and Bernard F. McKenzie.* Mayo Fndn. and Mayo Clinic, Rochester, Minn.

The frequent association of nephrosis with septic sore throat interested us in studying the influence of streptococcus toxin on canine experimental nephrosis. Healthy dogs were divided into 3 groups. One group was given nephrotoxic serum prepared from rabbits sensitized against dog kidney. Another group was given a streptococcus culture filtrate, prepared by filtering a 4-day culture in veal infusion broth of Streptococcus pyogenes C203M through a Mandler candle. A 3rd group was given a mixture of nephrotoxic serum and streptococcus culture filtrate. The total dose of each of the nephrotoxic serum and the streptococcus culture filtrate was 4.4 ml/kg of body weight divided into 9 doses and given 4 times daily over 3 days. In the combination of 9 ml of streptococcus culture filtrate was added to the total dose of nephrotoxic serum, making 1 ml of filtrate in each of the 9 doses. The plasma protein, cholesterol, blood urea and urinary proteins were quantitated before, during and after the period of administration of the nephrotoxic agents until the dogs practically recovered. Intravenous nephrotoxic serum alone produced severe hypoproteinemia, severe proteinuria, hypercholesterolemia, elevation in blood urea and some evidence of fluid retention. Intravenous streptococcus culture filtrate produced similar but much milder changes and the dogs recovered much faster. Intravenous administration of a mixture of nephrotoxic serum and streptococcus culture filtrate produced a clinical picture and blood and urinary findings similar to those produced by nephrotoxic serum alone. However, the combination (NTS+ ST) caused a slightly higher peak in urea retention and hypercholesterolemia, which was of longer duration. The proteinuria disappeared on the 20th day after administration of nephrotoxic serum alone, while the dogs given both nephrotoxic serum and streptococcus culture filtrate exhibited persistent proteinuria which was still present even on the 42nd day after the last injection.

579. FIXATION OF 5-HYDROXYTRYPTAMINE BY BRAIN MITOCHONDRIA. E. J. Walaszek* and L. G. Abood. Div. of Psychiatry, Univ. of Illinois College of Medicine, Chicago.

As part of a general program to investigate the possible role of 5-hydroxytryptamine (5-HT, serotonin) and other neurohumoral amines in neural function, it has been found that most of the 5-HT present in rat brain is bound to mitochondria. Mitochondria, which were isolated in isotonic (0.25 M) sucrose by differential centrifugation, contained 60-75% of the total 5-HT in rat brain while the remainder was distributed among the nuclear-debris and supernatant fractions. As determined by bioassay on the rat uterus, the amount of 5-HT in adult rat brain was 200-250 $\mu\mu g$/gm wet weight; 5-HT could be released from mitochondria by a variety of means: prolonged exposure to distilled H_2O or mild detergents; repeated freezing and thawing; exposure to 10^{-5} M reserpine (in sucrose) for 10 min at 0°. When either untreated or reserpine-treated mitochondria are exposed to 5-HT, the 5-HT content rises from 20 $\mu\mu g$/mg mitochondrial N to a maximal value of 60 $\mu\mu g$/mg N. Insofar as reserpine was found to be an effective inhibitor of oxidative phosphorylation (P/O) at 10^{-5} M, a number of other "uncoupling" agents were tested for their ability to release 5-HT from mitochondria. While reserpine was the only agent studied which released 5-HT, phenylether (5×10^{-6} M) and, to some extent, Dibenamine, both potent uncoupling agents, effectively blocked the ability of mitochondria to bind 5-HT, Dinitrophenol, morphine, eserine and LSD-25 were ineffective. It would appear from these studies that P/O is not dependent on the presence of 5-HT nor is P/O essential for the binding of 5-HT by mitochondria. Although structural integrity of mitochondria is important for the fixation of 5-HT, there are reasons for believing that actual binding sites exist within mitochondria and that 5-HT is not simply retained by a mitochondrial "membrane." (Supported by Office of Naval Research and Dept. of Public Welfare, Illinois.)

580. PSEUDOBIGEMINY INDUCED BY KCl IN THE ADRENALECTOMIZED DCA-TREATED RAT. Sheppard M. Walker and Ava Wolf.* Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.

Fourteen mature male rats of the Anheuser-Busch strain were adrenalectomized, placed on a low K diet and injected with 2 mg daily of desoxycorticosterone acetate (DCA). About 4 wk after the operating the animals were restrained in a supine position and injected intraperitoneally with 800 mg/kg of 2% KCl solution. Electrocardiograms with standard limb leads and with leads directly from the right auricle were recorded with a Grass Model III-D electroencephalograph. About 30 min after the injection of KCl irregular dropping out of ventricular beats occurs. Shortly thereafter, regular 3:2 A-V block appears. The first beat of each group of 3 shows a normal P-R interval and QRS complex. The second beat shows a prolonged P-R interval and an abnormal QRS complex. In the third beat A-V conduction is blocked. The bigeminal heart action thus produced resembles true ventricular bigeminy. This bigeminal action may persist several minutes before ventricular fibrillation or ventricular standstill occurs. The P wave persists for several minutes after ventricular electrical records have disappeared. (Supported by research grant H-697(C6) from the Natl. Heart Inst., PHS.)

581. POTENTIALS EVOKED IN OLFACTORY BULB BY ELECTRICAL STIMULATION OF CONTRALATERAL BULB. Raymond R. Walsh (intr. by F. X. Gassner). Dept. of Physiology, Univ. of Colorado, Denver.

A single, monophasic pulse of short duration delivered to one olfactory bulb produces a negative potential in the contralateral bulb. The evoked potential has at least two components as distinguished by the latencies of their respective peaks. The peak of the first occurs about 6 msec and the peak of the second occurs approximately 14 msec after the stimulus. The duration of the total evoked potential is long—lasting about 25-30 msec. The potential is abolished or greatly reduced in magnitude after a parasagittal lesion is made in the anterior aspect of cerebrum. This latter observation seems to be a physiological confirmation of the anatomical studies which indicate that there is a direct pathway from each olfactory bulb to its paired member via the anterior commissure. (Supported in part by a grant from the Natl. Inst. of Neurological Diseases and Blindness, PHS.)

582. CHRONIC STRIATAL CATS. G. H. Wang, Labs. of Psychobiology and Otolaryngology, Johns Hopkins Hosp., Baltimore, Md.

The purpose of this work is to find out the functions of the corpus striatum by comparing the behavior and reflexes of chronic striatal cats with those of chronic thalamic cats. We prepared 3 chronic thalamic and 4 chronic striatal cats. Removal of either the forebrain or the cerebral cortex alone was done by subpial suction and at two stages. These two types of preparations show the following differences in behavior and reflexes. In behavior: 1) striatal cats clean their fur

regularly whereas thalamic cats do not; 2) striatal cats eat spontaneously whereas thalamic cats have to be fed; 3) striatal cats exhibit no sham rage whereas thalamic cats are notorious for it; 4) striatal cats appear to be much less active than thalamic cats. In reflexes: striatal cats show permanent exaggeration of the stretch reflex in the extensors of 4 extremities whereas thalamic cats never do so. These differences in behavior and reflexes between striatal and thalamic preparations indicate that, contrary to the current theory, the corpus striatum is capable of functioning alone, independently of the cerebral cortex. The functions of the corpus striatum may well be 1) maintenance of the proper sequence in the occurrence of the reflexes required in such an act as cleaning or eating and 2) inhibition, as first demonstrated by Mettler and his associates in 1939. Striatal inhibition of the facilitatory reticular system and "motor centers" in the interbrain and midbrain may be responsible for absence of sham rage, reduction of spontaneous activity and exaggeration of the stretch reflex in the extensors of extremities in striatal cats.

583. ELECTRONIC SERVO REGULATION OF ARTERIAL PRESSURE IN THE INTACT DOG. Homer R. Warner (intr. by Howard B. Burchell). Dept. of Physiology, Univ. of Utah and Latter Day Saints Hosp., Salt Lake City.

A rise in pressure within the carotid sinus results in an increase in the frequency of impulses transmitted by the carotid sinus nerve. Further, the fall in pressure that results from electrical stimulation of the central end of this nerve has been shown to be a function of stimulus frequency. These facts suggested the possibility of stimulating the function of the carotid sinus with an electronic circuit whose input is the signal voltage from a transducer sensing the arterial pressure and whose output is an intermittant square wave voltage (12 volts for 0.1 msec.) whose frequency depends upon the magnitude of the input voltage. If this output signal were applied to the central end of the carotid sinus nerve, a servo loop would be established. The arterial pressure would be dependent upon the setting of a potentiometer which controls the reference voltage. The accuracy of the pressure regulation obtained would, in part at least, be dependent upon the amount of amplification given to variations in input signal. Such a device has been constructed and tested on dogs anesthetized with Nembutal (30 mg/kg). Experience to date indicates that the principle is sound and the method is a useful means of controlling this variable in experimental animals. The range over which pressure may be so regulated is limited by the activity of the animal's own regulating system, but can be extended by bilateral vagotomy and carotid sinus denervation.

584. THERMOREGULATION IN THE ATROPINIZED SUBJECT. Paul Webb. Aero Med. Lab., WADC, Wright-Patterson AFB, Ohio.

Administration of atropine parenterally has been found to be effective as a means of achieving a sweat-free state in a resting subject in heat. The thermoregulatory capacity of such a preparation is greatly reduced, not only because of inability to sweat, but also because of reduced adjustability in vasomotor tone. Body temperature is therefor strongly influenced by the environmental conditions. Experiments will be reported in which fully atropinized subjects were exposed to cold, comfortable and warm environments, and their thermoregulatory responses evaluated.

585. ENZYME STUDIES IN MOUSE DYSTROPHIA MUSCULARIS. I. M. Weinstock,* Sandra Epstein* and A. T. Milhorat. Dept. of Medicine, Cornell Univ. Med. College, New York Hosp., New York City.

The discovery of a primary muscle myopathy of hereditary etiology in an inbred strain of mice (Michelson et al. Proc. Natl. Acad. Sciences 41:1079, 1955) has made available another approach to the problem of clinical muscular dystrophy. Using muscle from this strain of mice we have investigated the activity of several enzyme systems previously studied in other forms of muscular dystrophy. As in other forms of dystrophy, genetically dystrophic mouse muscle shows a decrease in total and non-collagen protein nitrogen and an increase in the absolute and relative amount of collagen nitrogen per gram net weight of tissue as compared to controls. Dry weight is unchanged. Aldolase activity of dystrophic mouse muscle is markedly less than that of controls whether enzyme activity is based on wet or dry weight, or non-callagen protein nitrogen. Cathepsin and cytochrome oxidase levels of dystrophic muscle are distinctly elevated over those of the controls when based on any of the above 3 standards of muscle mass. Succinoxidase activity/gm wet weight or/mg dry weight of muscle is essentially unchanged, but is increased in the dystrophic muscle when based on mg of non-collagen protein nitrogen. (Supported by grants from Muscular Dystrophy Assocs. of America, Inc.)

586. MECHANISM OF COUGH REFLEX. Robert J. Weir, Jr. (intr. by Ivor Cornman). Hazleton Labs., Falls Church, Va.

The purpose of this research was to study the neurological and mechanical dynamics of cough in dogs. The cough, produced by stimulation of the respiratory mucosa, the vagus nerve and branches of the vagus nerve, showed these structures to be the afferent pathway of the cough reflex. Cough was blocked to stimulation of the tracheal mucosa by abolition of these pathways thus conclusively demonstrating the afferent pathway for the cough reflex. Through threshold studies on the airway, a gradient to stimulation of the cough response was demonstrated. The carina and epiglottis were the most sensitive areas of the airway. An area 4 inches above the carina and the area of the secondary bronchi were the least sensitive. The vagus nerve was approximately 3 times more sensitive than the carina or epiglottis. The studies on the cough dynamics indicated that the functional presence of the glottis was not a necessary adjunct to the cough response, but that the high intrathoracic pressure of the cough resulted from the amplitude and rapidity of response of the respiratory musculature. Cough was found to be a modification of normal respiration.

587. SEVERE COLD-EXPOSURE OF LARGE RATS. A. Kurt Weiss (intr. by G. C. Ring). Dept. of Physiology, Univ. of Miami School of Medicine and Howard Hughes Med. Inst., Miami, Fla.

Exposure of 150-gm male Holtzman rats to 5°C for 5 days or longer results in elevated oxygen consumption rates of preparations from the liver, skeletal muscle, diaphragm, cardiac muscle and kidney cortex. When this experiment was repeated with larger and older rats weighing 300 gm or more, no changes were discernible in any of the tissues studied except the liver. Prolongation of cold-exposure to one month did not change the oxygen consumption rates of tissues other than the liver. When large rats were exposed to -5°C they were unable to withstand the adverse climat

the mean survival time was around 9 days. The oxygen consumption rates of several tissues from animals which had survived at -5°C for 10 days were measured. The liver was the only tissue studied whose metabolic rate was significantly elevated. Brown-Grant believes that thyroid stimulation does not take place in rats exposed to severe cold. Thus, if one accepts this hypothesis, the stimulation of the metabolic rate of liver under conditions of severe cold-exposure must be through some other mechanism. (Supported in part by research grant RG-4150(C2) from the Natl. Insts. of Health, PHS.)

588. PERFORMANCE AND FOOD INTAKE AT LOW TEMPERATURES. Bernard Weiss (intr. by E. K. Marshall, Jr.). Div. of Clin. Pharmacology, Dept. of Medicine, Johns Hopkins School of Medicine, Baltimore.

Two experiments were performed. In the first, 30 rats were exposed for 20 min daily to temperatures of 0°, 10° and 20°C, in a box that contained a lever that could be pressed by the rat to deliver a pellet of food. Feeding was allowed otherwise for 1 hr daily, directly after exposure. Each animal was exposed to each temperature during each 3-day experimental period. The experiment was continued for 24 days, or 8 periods. The food intake data show an initial depression at 0° and 10°. After the third period, however, food intake rankings are restored to what would be expected on the basis of metabolic cost. The performance data also show a sharp initial inhibition and subsequent rise. The second experiment used temperatures of -10° and 20°, with 30 rats alternating for 6-day periods between these. Both performance and food intake data show a steep rise for the first few days and then a leveling off. Thus, both performance and metabolic adjustment criteria reveal not only that acclimatization arises from brief exposures, but that these are highly specific to temperature. (Studies performed in the labs. of the Dept. of Exptl. Psychology, USAF School of Aviation Medicine.)

589. RELATIONSHIP AMONG PLASMA CHOLESTEROL, AORTIC CHOLESTEROL AND BLOOD PRESSURE IN THE HEN. H. S. Weiss,* R. K. Ringer* and P. D. Sturkie. Lab. of Avian Physiology, Dept. of Poultry Science, Rutgers, State Univ. of New Jersey, New Brunswick.

Plasma cholesterol and direct blood pressure measurements were made on 32 hens, 1-2 years of age. After sacrifice, gross atherosclerotic scores and cholesterol levels were determined separately on thoracic and abdominal segments of the aorta. Groups of these birds had been treated either with estrogen, cholesterol or adenosine-5-monophosphate. Although the cholesterol and estrogen tended to elevate plasma cholesterol and the AMP to decrease aortic cholesterol, no significant difference were detected among the small groups used. Therefore all groups were pooled and treated as one sample in subsequent comparisons. Abdominal aortic cholesterol averaged 38.5 mg/gm and was significantly higher than the 26.8 mg/gm of the thoracic segment. The correlation between sclerosis score and tissue cholesterol was not significant for the thoracic segment ($r = -.06$), but was significant ($p = .05$) for the abdominal segment ($r = +.36$). Plasma cholesterol was not significantly correlated with the thoracic or abdominal aortic cholesterol levels ($r = +.29$) and $+.24$ respectively). None of the pressures was significantly related to thoracic aortic cholesterol. Highly significant correlation coefficients ($P < .01$) were found between pulse and systolic pressure on one hand and abdominal aortic cholesterol ($r = +.46$ and $+.52$), but not for diastolic pressure ($r = +.24$). Thus it appears that atherogenic changes in the aorta of the hen may produce measurable hemodynamic responses.

590. EFFECTS OF POSTURE AND ATROPINE ON CARDIAC OUTPUT. Arnold M. Weissler,* James J. Leonard* and James V. Warren. Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.

As part of the study of the circulatory adjustments to the upright posture, the effects of atropine on subjects in the horizontal and passive upright posture have been observed. Three groups of normal male subjects were studied before and 2 min after the intravenous injection of atropine sulfate, group A (6 subjects) in the recumbent position, group B (6 subjects) at 60° head-up tilt and group C (4 subjects) at 60° head-up tilt during sustained antigravity suit inflation (5 min at 70 mm Hg of body pressure). In group A striking increases in cardiac output (dye technique) were noted in all subjects (average 74% above the mean control cardiac index of 2.9 l.). These changes in output were accompanied by tachycardia with little or no change in stroke volume. Mean arterial pressure rose slightly (mean change +7 mm Hg). In contrast, the subjects in group B demonstrated only slight increases in cardiac output (average 9% above the mean control index of 2.6 l.), marked increases in pulse rate and diminution in stroke volume. Mean arterial pressures were not significantly altered. Sustained anti-gravity suit inflation in the head-up posture was found to elevate the cardiac output slightly. The response to atropine under these circumstances was similar to that in recumbent subjects. These data demonstrate a diminished cardiac output response to atropine during 60° head-up tilt and suggest a more general limitation of cardiac responsiveness in the passive erect posture.

591. QUANTITATED EXPERIMENTAL AORTIC REGURGITATION AND EFFECT OF MITRAL REGURGITATION. G. H. Welch, Jr., E. Braunwald, S. J. Sarnoff (intr. by A. J. Derbyshire). Lab. of Cardiovascular Physiology, Natl. Heart Inst., Bethesda, Md.

A canine preparation was devised in which the circulatory effects of aortic regurgitation could be acutely produced and quantitated. Blood was permitted to flow from the descending thoracic aorta into the left ventricle via a Potter turbine flowmeter through a tubular prosthesis sutured into the apex. Observations on the circulatory effects of concomitant mitral regurgitation were made possible by the use of a side-arm from the left ventricular prosthesis leading to the left atrium. Continuous recording was made of forward flow, regurgitant flow, heart rate and pressures in the left atrium, left ventricle and aorta. Aortic regurgitant volumes could be regulated by grading the size of the regurgitant bylpass orifice. Aortic regurgitant flows ranging from zero to values in excess of the dog's basal cardiac output were produced. The effective left ventricular function curves were depressed during aortic regurgitation. With marked aortic regurgitation left ventricular end-diastolic pressures were elevated and substantially exceeded left atrial pressures. When mitral regurgitation was then added in the presence of the fixed aortic regurgitant orifice, left atrial pressure rose, left ventricular end-diastolic pressure fell, while aortic regurgitant flow increased markedly. It may be concluded that the aortic regurgitant flow is limited by a competent mitral valve and conversely

that associated mitral regurgitation augments aortic regurgitant flow.

592. EFFECT OF TOBACCO SMOKE ON THE ARTIFICIALLY INDUCED VAGINAL CYCLE IN SPAYED RATS. W. A. Wellband, Jr.,* Nancy Miner* and A. H. Steinhaus. George Williams College, Chicago, Ill.

The effect of exposure to cigarette smoke on the vaginal response, artificially induced by an estrogen, was studied in 8 ovariectomized Sprague-Dawley rats. The dosage of estradiol was reduced to the lowest amount capable of consistently producing a typical estrual smear. A series of 3 injections (later reduced to 2) of 0.05 μg, each in oil, was administered during a 24-hr period approximately every 7th day. Four animals were then placed for 74 days in a chamber that was automatically filled each hour with smoke-laden air from a cigarette. The controls were housed in an identical chamber supplied with a change of fresh air hourly. Excluding individual differences and minor variations from cycle to cycle, the vaginal responses of the animals subjected to smoke were similar in every way to those of the controls in fresh air.

593. RELATIONS OF DRIVE AND REWARD SYSTEMS IN THE HYPOTHALAMUS. Richard Wendt and James Olds (intr. by D. B. Lindsley). Univ. of California and VA Hosp., Los Angeles.

Attempts were made to find points in the hypothalamus and adjacent forebrain regions were electric stimulation would produce increments and decrements in various kinds of eating behavior. Sites of these effects were studied a second time to determine whether electric stimulation would be rewarding or punishing in its effect on Skinner box behavior. Electrodes were chronically implanted in about 25 rats by means of a stereotaxic instrument and an electrode holder was screwed to the skull to hold them firmly in place. After the animal recovered from the operation tests of stimulation effects on eating behavior were made first. Animals were maintained on an ad lib. diet in the home cage. For 1 hr a day, animals were stimulated with a 60-cycle sine wave current for half a second every 10 sec. During this period they were in boxes with food available and food consumption during the period of stimulation was measured. This measure was compared with a baseline measure taken during similar periods the week before and the week after the stimulation tests. In general it did appear that some electrode placements in the lateral hypothalamus produced increase in eating behavior; and some in placements just medial to these produced some decrease in eating behavior. Later self-stimulation tests were made on the same animals. They were placed in the same boxes, this time with a pedal. They could step on the pedal to deliver a stimulus up to half a second to their own brains. Under these conditions, some animals stimulated their brains over 500 times an hour. Others avoided the pedal, stimulating their brains not more than once or twice by accident. Others stimulated themselves at a chance rate. From these tests it appears that there is quite a tendency for animals to avoid the stimulation of the lateral hypothalamus that increases eating behavior. And there seems to be quite a tendency to pursue avidly the stimulation by more medially placed electrodes which decrease eating behavior.

594. RELATION OF SALURESIS OF UREA LOADING TO NORMAL EXCRETION OF ELECTROLYTE. Clark D. West and Ruth K. Bayless.* Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, Ohio.

Electrolyte excretion under normal conditions has been compared with that occurring during urea loading to ascertain whether, under the 2 conditions, similar parameters of excretion might exist which would explain the saluresis accompanying loading. Both hydropenic and hydrated dogs were used. After 3 periods of urine collection, urea was infused at a slow rate to produce a 3- to 4-fold increase in total solute excretion. The data have been compared with those for experiments performed identically except that the solute load was omitted. Earlier studies suggested that administration of an additional solute load to an animals already undergoing solute diuresis enhanced the saluresis without change in the ratio of sodium or chloride to total solute. A similar phenomenon could be demonstrated in passing from the non-loaded to the loaded state in the present experiments, providing the solute load was administered slowly. Thus in hydrated animals, ratios of sodium, chloride and potassium to total solute were the same in the course of the loading as in the control experiments despite significant differences in rates of excretion. In hydropenic dogs, the ratios for chloride and potassium behaved as in hydrated animals, but those for sodium increased significantly with loading. The ratios for ammonia, titratable acid, bicarbonate and phosphorus, on the other hand, fell with loading. Under the conditions of the study the saluresis of loading appears for the most part to be merely a magnification of normal electrolyte excretion, the degree of magnification depending on the increment in total solute excretion.

595. INHIBITORY REFLEXES ELICITED BY INTRACORONARY INJECTIONS OF ACETYLCHOLINE, VERATRIDINE AND GANGLIONIC STIMULATING AGENTS. J. W. West and S. V. Guzman (intr. by C. F. Schmidt). Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, and Div. of Cardiology, Philadelphia Gen. Hosp., Philadelphia, Pa.

In dogs under morphine-chloralose anesthesia the circumflex or anterior descending branch of the left coronary artery was catheterized fluoroscopically via one carotid artery. The chest remained intact. Records were made of: a) leg perfusion pressure while maintaining leg blood flow constant with a Dale-Schuster pump; b) electrocardiogram; c) pneumogram and d) femoral arterial blood pressure. Following control measurements, acetylcholine (0.8-4.0 μg/kg), DMPP (0.2-2.0 μg/kg), nicotine (0.2-2.0 μg/kg) and veratridine (0.04 μg/kg) given by intracoronary injection produced the full Bezold-Jarisch reflex (respiratory inhibition, bradycardia, hypotension and a decrease in leg perfusion pressure). These effects were eliminated by bilateral vagotomy. Atropine (0.1 mg/kg) intravenously abolished the bradycardia but not the respiratory inhibition, hypotension and decrease in leg perfusion pressure. Tetraethyl ammonium bromide (0.01-0.1 mg/kg) injected intra-arterially blocked or greatly diminished the immediate response to intra-arterial injections of acetylcholine, DMPP and nicotine but did not block the immediate response to intra-arterial injection of veratridine, even when tetraethyl ammonium bromide was given in doses from 0.1-100 mg/kg. Intracoronary arterial injection of mecholyl (0.8-4.0 μg/kg) did not produce the Bezold-Jarisch effect.

596. RELATION OF SUCCINATE TO ADRENOCORTICAL FUNCTION. William L. West* and Walter M. Booker. Dept. of Pharmacology, Howard

Univ. Med. School, Washington, D. C.

We have reported that succinate-treated animals when subjected to cold stress survive poorly during the stress as when compared with their treated controls. This "succinate effect" may be antagonized by the administration of cortisone. Likewise cortisone in high concentrations antagonizes the succinate oxidase system of liver homogenates in phosphate buffer pH 7.45. You and Sellers (1951) have shown that during cold stress the succinate oxidase system increases. We have suggested that coritsone acts to slow down the increased demand on the metabolic reserve or the increased cycling of the Krebs citric acid cycle during stress. We thought it would be of interest to determine the "specificity" of the cortisone antagonism of the succinate oxidase system. We have also studied cortisone effect on liver succinate oxidase system in 0.27 Nl sucrose and recorded P/O ratios. Cortisone inhibits the succinate oxidase system and uncouples oxidation and phosphophylation.

597. A RESPIRATION-TYPE CHAMBER FOR LONG-TERM TOTAL ENERGY METABOLISM STUDIES IN MAN. G. Donald Whedon,* Ernest E. Huber, Jr.,* Ronald H. Thompson* and Russell M. Wilder. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

A life-long interest in study of effects of activity and disease upon human total energy metabolism has led to development of a metabolic chamber large enough to permit varied ambulatory activity. Energy expenditure is measured by a technique which affords continuous analysis and recording of expired air for minute-to-minute variations in oxygen, carbon dioxide and relative humidity over several hours or days. Studies are intended of activity metabolism and work efficiency and of environmental, hormonal and nutritional influences on metabolism in normal and diseased states. The chamber is air conditioned for close control over a range of 5-49°C and 10-95% relative humidity over most of these temperatures. The subject wears a light "space" helmet of optical quality plexiglass and flexible vinyl, in which he sleeps, eats, walks and works. The helmet is connected by flexible tubing via the chamber wall to the analyzer train, meter and pump. Oxygen concentration is determined by a pair of modified Pauling para-magentic analyzers sharing a power supply regulating voltage to ±0.0004%; drift under working conditions is 0.1% in 24 hr. Relative humidity is measured by electric hygrometers. Carbon dioxide is determined in a pair of infra-red bolometer stream analyzers which have been linearized. Resolution and response time of the continuous analysis system are such that yawning and talking are readily reflected in the records.

598. RESPONSES OF MAMMALS TO PARTIAL DEPLETION OF EXTRACELLULAR ELECTROLYTES. Wycliffe Wight* and Robert E. Semple. Dept. of Physiology, Queen's Univ., Kingston, Canada.

The effects of extracellular electrolyte depletion of peritoneal dialysis with isotonic glucose solution were observed in rats, rabbits and dogs. Conditions were the same for all animals. After dialysis sodium chloride was removed from the diet for 5 days and was then restored. Daily measurements were made of body weight, food and water intake and the amounts of sodium, potassium, chloride and nitrogen excreted in the urine. Hematocrits were measured at appropriate intervals as were concentrations of sodium, potassium, chloride and nitrogen in plasma and dialysates. In all animals dialysis resulted in immediate and significant decreases in electrolyte concentrations in plasma and increases in hematocrit. In the rats and rabbits electrolyte concentrations and hematocrits were back near control levels 24 hr later, but return to control fluid composition took longer in the dogs. During the period of salt depletion excretions of electrolytes and water were all greater than the corresponding intakes and body weights decreased. Restoration of sodium chloride resulted in positive electrolyte and nitrogen balances with increases in body weight. The immediate increases in hematocrit concentration that follow dialysis may be the result of a shift of fluid from the extracellular to the intracellular compartment but the changes in electrolyte concentrations of plasma do not indicate immediate fluid shifts. Glucose concentrations of plasma increase greatly after dialysis with either glucose or Locke's solution indicating that stress may play a significant role in some of the changes observed. (Supported by the Natl. Research Council of Canada.)

599. CREATINE METABOLISM IN RATS FOLLOWING WHOLE BODY IRRADIATION. Clyde M. Williams, George M. Krise, Donald R. Anderson and Robert M. Dowben (intr. by Henry M. Sweeney). USAF, School of Aviation Medicine, Randolph AFB, and the Radiobiological Lab., Balcones Research Ctr., Austin, Tex.

A creatinuria, approximately 20 times normal values, occurs in rats after irradiation by 1500 r of cobalt-60 γ-rays. The creatinuria is greatest during the 2nd or 3rd day after irradiation and lasts for 5 or more days. Concomitantly there is about a 3-fold increase in plasma creatine levels; 435 r of mixed radiation (fast neutrons—γ-rays, 3:1) resulted in a 36-63 fold creatinuria on the 2nd post-radiation day. In all animals that died, the creatinuria did not abate after a few days but continued to rise until death, reaching levels as high as 85 times normal. A creatinuria did not appear in pair-fed controls. No change was observed in the excretion of creatinine and guanidoacetic acid in the urine of irradiated animals. Post-radiation elevation of serum glutamic-oxalacetic transaminase was found. Experiments with carbon-14 labeled creatine indicate that the excreted creatine comes from the body pool of creatine.

600. ENHANCED OXIDATION OF CARBOHYDRATES IN ADRENALECTOMIZED RATS STUDIED WITH 2-C^{14} PYRUVATE. William W. Winternitz* and Daniel L. Kline. Dept. of Physiology, Yale Med. School, New Haven, Conn.

Previous studies have demonstrated a deficit of carbohydrate in adrenalectomized rats following epinephrine (Proc. Soc. Exper. Biol. & Med. 81:683, 1952). The respiratory quotients suggest that the extra carbohydrate is oxidized (Fed. Proc. 15:202, 1956). These experiments have been extended using C^{14}-labeled pyruvate as a tracer. The adrenalectomized rats showed an increased amount of expired C^{14} carbon dioxide as compared to normal controls. This is consistent with the view that the adrenal cortical hormones inhibit the oxidation of carbohydrate. These isotope experiments in conjunction with the respiratory studies make it even more certain that enhanced carbohydrate oxidation in the adrenalectomized rat accounts at least partially for the previously observed carbohydrate deficit.

601. GROWTH HORMONE AND NITROGEN RETENTION IN THE HYPOPHYSECTOMIZED RHESUS MONKEY. R. C. Wolf,* E. Knobil and R. O. Greep.

Biological Research Lab., Harvard School of Dental Medicine, and Dept. of Physiology, Harvard Med. School, Boston, Mass.

The effects of equipotent growth hormone preparations obtained from beef (Armour) and monkey pituitary glands (Wilhelmi) on nitrogen balance of hypophysectomized rhesus monkeys were investigated. Purified monkey growth hormone returned the rate of nitrogen retention to the prehypophysectomy level in contrast to beef growth hormone which was without effect (Endocrinology 60:166, 1957). Subsequent experiments have shown significant increases in nitrogen retention within 24 hr following one intramuscular injection 5 mg/kg of monkey growth hormone; with 1 mg/kg monkey growth hormone daily, nitrogen retention became evident 72 hr after the first injection. In some instances, injection of the higher dosage resulted in symptoms of "toxicity" within 5-7 days. These symptoms consisted of moderate to severe edema and anorexia, the latter resulting in a nitrogen loss. Discontinuation of treatment was necessary. In one case, growth hormone was re-administered 7 days following the initial interruption of treatment and the animal responded, as before, by an increase in nitrogen retention suggesting absence of refractoriness. The cessation of growth hormone injections in those instances where untoward symptoms did not appear was followed by a return of nitrogen balance to control levels within 24-48 hr. At no time did beef growth hormone produce significant effects on nitrogen balance or the well-being of the hypophysectomized monkeys.

602. HYPOCHOLESTEROLIZING EFFECT OF EXERCISE ON CHOLESTEROL-FED COCKERELS. Harry Y. C. Wong, Merton B. Anderson,* John K. Kim,* David J. Liu* and Edward W. Hawthorne. Depts. of Physiology and Pathology, Howard Univ. College of Medicine, Washington, D. C.

Previous studies in our laboratory have shown that exercise in cholesterol-fed cockerels treated with testosterone propionate resulted in a significant decrease of the cholesterol level of the blood and a subsequent reduction in the formation of atherosclerosis of the abdominal aorta of young cockerels (Fed. Proc. 15:659, 1956). Further studies were undertaken to study the effect of exercise on cholesterol-fed cockerels. Three groups of cockerels were studied: 1) controls, on plain mash; 2) cholesterol-fed only; and 3) similarly treated as group 2 with the exception that these birds were exercised twice daily for 7 wk. The "atherogenic" diet was 2% cholesterol and 5% cottonseed oil mixed with mash. After 7 wk on this regimen, it was observed that there was a significant difference in the cholesterol level of the blood of group 1 when compared to groups 2 or 3 and a marked difference when group 2 was compared with group 3. In addition, there was a subsequent reduction in the formation of atheromatous plaques of the abdominal aorta of the exercised birds when compared with the cholesterol-fed only. Our data also indicate that there was a marked difference in the adrenal weights of the cholesterol-fed only and the exercised birds when compared to the controls. However, there were no differences in the testes weights of any of the groups studied. (Supported by Research Grants, # H-2420, Natl. Heart Inst., PHS, Committee on Research, Council on Pharmacy and Chemistry, A.M.A. and the Washington Heart Assoc., Washington, D. C.)

603. FUNCTIONAL LOCALIZATIONS IN THE AMYGDALA. Charles D. Wood and Byron A. Schottelius (intr. by Walter S. McClellan). Dept. of Physiology, Univ. of North Carolina, Chapel Hill.

Blood pressure and respiratory responses produced by stimulation of the cortico-medial and baso-lateral amygdala in rats and cats were observed. Responses were variable with light anesthesia. With deeper anesthesia a pronounced fall in blood pressure and inhibition of respiration were produced by stimulation of the cortico-medial amygdala. Although higher voltages were employed these responses were less pronounced in the baso-lateral area. Apnea was produced with initiation of stimulation, but respiration was reestablished after 5-10 sec even though the stimulus was continued. After bilateral lesions of the ventro-medial hypothalamic nuclei the blood pressure responses to stimulation of the cortico-medial amygdala were abolished. The threshold for respiratory inhibition from the cortico-medial area was raised. Gloor (1955) has demonstrated that the cortico-medial but not the baso-lateral amygdaloid area project to the vento-medial hypothalamic nuclei. Rates with bilateral lesions of the cortico-medial area maintained for a period of 3 months demonstrated no chronic blood pressure changes or alterations of behavior. These results indicate that, at least in surgical levels of anesthesia, the cortico-medial amygdala exerts a greater influence on blood pressure and respiration than does the baso-lateral region. The blood pressure responses to stimulation of the cortico-medial area appear to be mediated through the ventro-medial nucleus of the hypothalamus, however, the fibers concerned with respiratory inhibition appear to be only partially involved with this structure.

604. POTASSIUM TRANSFER AND METABOLISM IN CARDIAC AND SKELETAL MUSCLE. J. C. Wood,* H. L. Conn, Jr. and M. E. Molthan.* Dept. of Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia.

An intimate relationship between muscle function and electrolyte composition is established. The dynamics of electrolyte behavior in muscle are relatively obscure. In these studies, potassium transfer kinetics and some other aspects of metabolism were studied in the isolated dog heart and resting skeletal muscle. The organs were perfused at a constant rate with arterial blood to which radiopotassium and other agents were added in a manner providing a perfusate of fixed, determinable composition. Intercompartmental steady-state transfer rates of potassium were derived by analyzing the approach of venous to arterial specific activity. Arterial and venous serum sodium and potassium, blood oxygen, carbon dioxide, pyruvic and lactic acid and muscle water, sodium and potassium were measured. Muscle QO_2, QCO_2, sodium and inulin spaces were derived. Values comparable to those reported by others were found for the various metabolic parameters investigated. All radiopotassium curves indicated a double exponential approach of venous to arterial activity. These curves and ancillary data seemed best analyzed within a 3-compartment series framework. In both types of muscle, calculated effective blood-interstitial space exchange rates appeared to be blood flow limited. Interstitial-intracellular space potassium exchange averaged about 1.0 mEq/kg/min in the beating, "nonworking" hearts and about 0.4 mEq/kg/min in resting skeletal muscle. The results are compatable with the concept of one or more modes of potassium exchange between cells and ISF in cardiac and skeletal muscle as studied, providing all pathways permit exchange with essentially all intracellular potassium.

605. MOISTURE INDEX, A NEW CLOTHING VARIABLE. Alan H. Woodcock and John R. Breckenridge.* Quartermaster Research and Develop. Ctr., Natick, Mass.

The introduction by Gagge, Burton and Bazett of the concept of thermal insulation in the quantitized form of the clo unit inaugurated the scientific approach to heat transfer problems of clothed man under cold stress. Since there has been no adequate quantitative index of clothing when man is under heat stress, studies of this type have been limited to either nude men or those clad in clothing which can be described in such quantitative terms as "light." A new index of the clothing system is proposed which, together with the insulation value in clo, will allow a quantitization of heat transfer property of clothing and which can be fitted into heat transfer equations of men under heat stress. By means of these two indices (clo and moisture index), upper and lower limits can be arbitrarily determined, of the range of temperatures in which clothed man can remain in thermal equilibrium at a given heat production rate. The moisture index is based on the conversion of difference in vapor pressure between skin and environment into an equivalent temperature difference which is added to the measurable temperature difference between skin and environment. Furthermore, with a knowledge of the insulation and moisture index of clothing, the increment of heat loss from the skin caused by a rise in either vapor pressure or temperature of skin can be predicted independent of environmental conditions.

606. AMPLIFICATION OF SYSTOLIC PRESSURE IN THE AORTIC-RADIAL SYSTEM IN MAN. J. Leo Wright (intr. by Norman M. Keith). Mayo Fndn. and Mayo Clinic, Rochester, Minn.

Systolic amplification in the aortic-radial system has been studied in normal adults and patients with cardiovascular disease by retrograde catheterization of the aorta via radial, brachial and femoral arteries, followed by graduated catheter withdrawals during simultaneous recording of radial and aortic pressure pulses. In 15 healthy adults the average increase in systolic pressure at the radial artery was 12% (range: 2-21%) (aorta: 126/81 mm Hg; range: 112/71-150/91) (radial: 142/77; range: 122/68-166/88). In 5 patients with aortic insufficiency systolic amplification at the radial artery averaged 24% (-1%-47%) (aorta: 162/56; range: 149/44-194/72) (radial: 201/52; range: 172/42-233/62). In 13 of 15 patients with severe aortic stenosis, systolic-pressure amplification was absent at the radial artery. In the group the average systolic-pressure decrease at the radial artery was -3% (range: -7%-4%) (aorta: 109/67; range: 77/53-142/85) (radial: 106/64; range: 80/55-133/82). In 3 patients with mitral stenosis and auricular fibrillation the systolic pressure decreased at the radial artery an average of -6% (range: -7% to -4%). When amplification of systolic pressure at the radial artery was absent, there was also a striking decrease in the degree of change in contour of the aortic pulse as it moved to the radial artery. These data demonstrate a relationship between the phenomenon of systolic-pressure amplification and the transformation in contour of the arterial pressure pulse as it moves peripherally in the aortic-radial system.

607. LIGHT-INDUCED TRANSIENT CHANGES IN THE ABSORPTION SPECTRUM OF RHODOPSIN IN SOLUTION. V. J. Wulff, E. W. Abrahamson,* Henry Linschitz* and R. G. Adams.* Syracuse Univ., Syracuse, N. Y.

During and following excitation with a 400-joule light flash of approximately 100 μsec duration, rhodopsin in solution exhibits a sequence of changes in its absorption spectrum. As early as 15 μsec after the onset of the flash there is a shift in the absorption maximum to 495 mμ, and enhanced absorption in the red and blue regions of the spectrum. These changes persist during the exciting flash. After the flash terminates the enhanced absorption in the red vanishes at about 200 μsec and the enhanced absorption in the blue is considerably reduced 500 μsec after onset of the flash. With the end of the flash there occurs a second but slower shift of the absorption maximum toward the blue. These shifts toward the blue and the decline in absorption in the red serve to produce a region of increased transmission (i.e. bleaching) which exhibits an initial maximum at 530mμ. One minute after the exciting flash the solution in the absorption cell is orange in color, which fades slowly upon further exposure to light. In the dark the orange pigment persists and exhibits two absorption maxima, one at 475mμ and one at 368mμ. The absorption spectra were photographed on spectrograph plates and the absorption curves were reconstructed from the spectra. The rhodopsin solutions were exposed to 2 consecutive flashes with a controlled time interval. The initial 400-joule, 100 μsec exciting flash was followed by a 14-joule, 15 μsec analysis flash which entered the slit of the spectrograph. The plates were calibrated for density and wave length and suitable control flashes were recorded.

608. REDUCTION OF ADRENAL CORTICAL STEROIDS BY PASSIVELY CONGESTED RAT LIVER. F. Eugene Yates and John Urquhart (intr. by E. M. Landis). Dept. of Physiology, Harvard Med. School, Boston, Mass.

To determine whether or not hepatic inactivation of adrenal coritcal steroids is impaired in congestive heart failure, some of the major circulatory changes associated with cardiac failure were stimulated in male rats. The inferior vena cava above the hepatic veins was constructed to less than 20% of its initial cross-sectional area with cotton ligatures. This procedure doubled peripheral venous pressure and severely diminished sodium and water excretion following intravenous infusions of saline though gross ascites was rate. On a high salt diet the venous-congested animals were more responsive than were control animals to the sodium-retaining effects of desoxycorticosterone acetate (DOCA), 1 mg/rat/day subcutaneously and developed positive sodium balance at the rate of approximately 1 mEq/rat/day while the controls retained only 0.5 mEq/rat/day. Adrenalectomized venous-congested rats maintained on DOCA survived significantly longer after withdrawal of the steroid than did control adrenalectomized rats. Possible impairment of steroid inactivation was investigated more directly ty testing the ability of sucrose homogenates of normal and passively congested rat livers to reduce the Δ^4-3-keto group of desoxycorticosterone, cortisone and hydrocortisone. The assay was designed so that reaction velocities were constant (zero order) with respect to substrate concentration and were linearly related to homogenate concentration at all levels of activity studied, making quantitative comparisons possible. The rate of reduction of the Δ^4-3-keto group in desoxycorticosterone was significantly diminished in homogenates of chronically congested livers (to 55% of control levels) but the inactivations of cortisone and hydrocortisone were unimpaired.

609. OXYGEN CONSUMPTION IN SKELETAL MUSCLE DURING REACTIVE HYPEREMIA. Lloyd R. Yonce (intr. by Philip Dow). Depts. of Physiology and Pharmacology, Med. College of Georgia, Augusta.

Reactive hyperemia is probably not neurogenic or histaminic in origin or due directly to the lactic or carbonic acid produced; but it is greater in amount and duration (within limits) the longer the period of occlusion. A new attepmt has been made to relate the oxygen consumption and the oxygen debt and its repayment in skeletal muscle to the degree and duration of vasodilation produced by temporary occlusion of the blood flow. The gracilis muscle of dogs was isolated by a mass ligature on either end and was separated from the skin and the underlying muscle. Blood flow was restricted to a single arterial supply and venous drainage. The venous flow was measured by a drop recorder working under oil to permit anaerobic collection of blood for analysis of oxygen content by a microgasometric method (Scholander and Irving). Compensation of the oxygen debt occurred by at least 2 responses, an increase in the arterial-venous oxygen difference and an increased blood flow (reactive hyperemia). The oxygen repayment was essentially completed during the period of hyperemia, suggesting that aerobic metabolism is a factor in the removal of the vasodilation. After the hyperemia there was a decreased blood flow. This may be an overshoot of the factors controlling the hyperemia and needs further investigation. Current experiments are concerned with the relation between the amount of hyperemia and the oxygen debt. (Supported by grants from the Natl. Heart Inst. and the American Heart Assoc.)

610. CLOSED-CIRCUIT TELEVISION FOR STUDY OF MICROCIRCULATION DURING CENTRIFUGATION. Paul L. Yudkofsky (intr. by Joseph F. Tomashefski). Aero Med. Lab., Wright Air Development Ctr., Wright-Patterson AFB, Ohio.

Although the circulatory disturbances resulting from exposure of man and laboratory animals to acceleration have been described, previous observations have been confined for the most part to gross changes in the cardiovascular system. To examine microcirculation during this type of stress, anesthetized golden hamsters (Mesocricetus auratus) were mounted on the U.S. Air Force human centrifuge and the small blood vessels of the cheek pouch were observed at high magnifications by means of a microscope and closed circuit television combination. The TV eye (RCA-M136250) modified to withstand the increased gravitational forces generated during centrifugation was connected through a control unit (RCA-M136251) to a standard television receiver. The camera lens was removed from the TV Eye and a fixed focus compound microscope tube was joined to the TV Eye by means of a metal collar. Standard microscope lenses and a concentrated arch lamp were used. The hamster preparation, attached to a movable stand for focusing and the TV Eye were mounted in the cab of the human centrifuge. The control unit was placed at the centrifuge axis and the television receiver was installed in another room. Magnifications of 430 x at the camera and 900 x on the screen were achieved with this apparatus during accelerations up to 12 "g's." The image is remarkably steady during multiple "g" runs at lower magnifications. Changes in vessel caliber and flow were observed and the TV image photographed.

611. PHOSPHATE LEVELS DURING AWAKENING FROM HIBERNATION. Marilyn L. Zimny (intr. by Louis A. Toth). Dept. of Anatomy, Louisiana State Univ. School of Medicine, New Orleans.

Changes in levels of metabolic compounds during hibernation in the ground squirrel (Citellus tridecemlineatus), showed a 74% increase for cardiac muscle phosphocreatine with a 72% decrease in adenosine triphosphate and for skeletal muscle phosphocreatine a 60% increase with a 55% decrease in adenosine triphosphate. Studies have been made of these compounds and glycogen levels in cardiac and skeletal muscle following a 15 minute and 30 minute awakening period after three to four days of uninterrupted hibernation. The results following the 15 minute awakening period are as follows: cardiac muscle- ATP, 49% increase; PC, 73% decrease and glycogen, 35% decrease; skeletal muscle- ATP, 26% decrease; PC, 54% decrease and glycogen, 35% increase. After the 30 minute awakening period; cardiac muscle- ATP, 82% increase; PC, 57% decrease and glycogen, 6% decrease; skeletal muscle- ATP, 13% increase; PC, 72% decrease and glycogen, 55% increase. The heart rate following the 15 minute arousal period is approximately 120 beats higher and that following the 30 minute arousal period 240 beats higher, than that during hibernation. Therefore a quick energy source must be available and cardiac muscle glycogen may be that source. Phosphocreatine may be the "trigger" source of energy for glycolysis during awakening from hibernation. (Supported in part by a research grant from the Louisiana Heart Association.)

612. ERRONEOUS MEASUREMENT OF PLASMA VOLUME BY T-1824 IN THE RABBIT. F. Zizza and E. B. Reeve (intr. by Cosmo G. Mackenzie). Dept. of Medicine, Univ. of Colorado School of Medicine, Denver.

Simultaneous measurements of plasma volume with T-1824 and I^{131}-albumin, prepared from rabbit albumin, have been made in 9 rabbits. The T-1824 and the I^{131}-albumin were injected from separate syringes and 3 or more samples were withdrawn over the next 30 min. The logarithms of the concentrations of T-1824 and I^{131}-albumin in the samples were plotted against the time of withdrawal and the concentrations at zero time were determined by extrapolation. The plasma volumes averaged 8.2% (S.D. 3.2) lower with I^{131}-albumin than with T-1824. Tests of the techniques used showed that: a) the volume of plasma in a beaker could be determined accurately with T-1824 or I^{131}-albumin; b) simultaneous measurements of plasma volume with T-1824 and red cell volume with P^{32}-labeled red cells gave an average value for "body hematocrit"/venous hematocrit of 0.84, very close to that reported elsewhere (Armin et al. 1952). The likely cause of the high values given by T-1824 is rapid removal of dye from the plasma immediately after injection. The loss rates of dye and I^{131}-albumin, respectively 25% and 15% during the 1st hr after injection, show that T-1824 is bound less firmly to albumin than the I^{131}. It is of interest that a revised value for "body hematocrit"/venous hematocrit calculated from the I^{131}-albumin measurement of plasma volume and the P^{32} measurement of cell volume, averages 0.89 and is close to that of 0.90 in the splenectomized dog and 0.91 in man.

613. PLATELET VISCOUS METAMORPHOSIS AND CLOT RETRACTION. Majorie B. Zucker and Jennie Borrelli.* Sloan-Kettering Institute, New York City.

Previous work has indicated that human thrombin will cause viscous metamorphosis (vm) of human platelets in the presence of albumin but not in saline

alone. Luscher and Bounameaux have shown that clot retraction will not occur in a saline medium, but proceeds in the presence of plasma dialysate or phosphate buffer with or without glucose. We have found that thrombin can similarly cause vm in the presence of plasma or albumin dialysate, of imidazole buffer, or of phosphate buffer pH 7.4 or 8.5, but not pH 6.5. EDTA, but not C -EDTA, inhibits vm and clot retraction produced by thrombin. However, retraction occurs when dialysed EDTA plasma is used. Only serums which evolved thrombin during incubation with platelets produced vm. Bergsagel's findings, however, suggest that PTC is important in producing vm. In order to test this, thrombin was prepared by Biggs and Macfarlane's method from the plasma of a patient with severe PTC deficiency. This thrombin, when added to washed platelets from a normal or PTC-deficient individual, produced a normal degree of vm in the presence of phosphate or albumin. Thus PTC does not seem to be essential in producing vm in this system. Surprisingly, thrombin from PTC-deficient blood caused much less clot retraction of normal or PTC-deficient platelet-rich ACD plasma (or citrated plasma to which washed platelets had been added) than did thrombin from normal plasma, although both were equal in their ability to clot fibrinogen or plasma.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Forty-eighth Annual Meeting

CHICAGO, ILLINOIS, APRIL 15-19, 1957

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614. EFFECTS OF CHOLESTEROL, CHOLIC ACID AND EFA DEFICIENCY UPON ADULT MALE RATS. Erik Aaes-Jørgensen* and Ralph T. Holman. Hormel Inst., Univ. of Minnesota, Austin.

Essential fatty acid (EFA) deficiency has been produced once in adult rats by severe caloric restriction. In this laboratory acceleration of EFA deficiency has been observed by dietary supplements which induce hypercholesterolemia. These techniques have now been applied to adult rats and, within 18 weeks, a mild deficiency condition was induced, judging from skin signs, growth and polyunsaturated acid contents of testes. Forty adult male rats (268 gm, av.) were divided into 4 equal groups and fed a diet free of EFA, ad lib. (diet 1). Three groups were fed diet 1 supplemented with 1% cholesterol (diet 2), 1% cholesterol + 0.5% cholic acid (diet 3) or 0.5% cholic acid (diet 4). After 18 wk, total weight gains were 180, 193, 181 and 158 gm, respectively. The average skin scores were 0.2, 1.0, 1.5 and 2.1, respectively, indicating light deficiency symptoms in the latter groups. At the 17th week, the right testes from 3 rats of each group were removed for polyunsaturated fatty acid analysis. The contents of polyunsaturated acids were least in the testes of rats fed cholic acid. Histological examination of the testes and epididymides revealed all except one to be normal. The exception was a rat fed cholesterol and the degeneration was severe. These results will be compared with similar data obtained on young male rats.

615. REACTION OF HYDROXYLAMINE WITH O-ACETYL GROUPS IN THE CELL WALL OF STREPTOCOCCUS FECALIS. Adolph Abrams. Dept. of Biochemistry, Univ. of Colorado School of Medicine, Denver.

While studying the reaction of hydroxylamine with various cell fractions of streptococcus faecalis prepared by sonic disintegration and differential centrifugation it was noted that the insoluble material gave a test for hydroxamic acid formation. The test was most intense in the fractions which sedimented between 1400-36000 x g for 15 min in 0.4 M sucrose. The hydroxamic acid was identified as acethydroxamic acid by paper chromatography in 4 different solvent systems. No other hydroxamic acid compound was observed. The acetyl group was alkali labile at room temperature, acid stable at room temperature and reacted slowly with hydroxylamine (0.66 M) at neutral pH but rapidly at very alkaline pH. These chemical properties indicate that the acetyl group is bound as an O-acetyl ester. The material to which the acetyl group is bound seems to be the cell wall. A soluble nondialyzable preparation made by treating the insoluble fraction with trypsin plus chymotrypsinogen gave the hydroxamic acid on treatment with alkaline hydroxylamine and contained rhamnose, glucose, amino sugar, glutamic acid, alanine and a basic amino acid (probably lysine). The soluble polysaccharide-peptide complex containing O-acetyl groups migrated as one electrophoretic band with a considerable negative net charge at pH 7.8. An insoluble preparation which was purified by repeated differential centrifugations and which was characterized by electron microscopy as being almost totally cell walls contained 1.2% O-acetyl groups whereas the whole cells contained 0.4% O-acetyl groups.

616. ADAPTIVE DEGRADATION OF HYDROXYPROLINE IN BACTERIA. Elijah Adams. Dept. of Pharmacology, New York Univ. College of Medicine, New York City.

Soluble extracts of soil bacteria grown on hydroxy-L-proline convert this compound to L-glutamate (Adams, Fed. Proc. 15:209, 1956). Intact cells appear to degrade hydroxyproline through glutamate as an intermediate, although the latter compound is not ordinarily excreted into the medium. Cells grown on hydroxy-L-proline, however, utilize exogenous glutamate only after an appreciable lag, its utilization without lag occurring only after cells have been exposed to it. These facts suggest adaptation to L-glutamate uptake as a factor limiting the rate of its utilization. The optical purity and chemical identity of L-glutamate formed from hydroxy-L-proline by extracts has been confirmed by isolation. Inhibitors (tetraborate or arsenite) which completely block the degradation of glutamate by extracts permit its accumulation in yields of 75% from either hydroxy-L-proline or allohydroxy-D-proline. The equally efficient formation of L-glutamate from either epimer is explained by the presence in crude extracts of a highly active epimerase which interconverts them. The epimerase appears adaptively only after growth on hydroxyproline and remains active in extracts on anaerobic incubation or after dialysis, conditions which prevent net hydroxyproline utilization. Of other amino acids tested (glutamic acid, proline, serine, alanine) only alanine is racemized by crude extracts and, unlike hydroxyproline epimerase, this activity appears after growth on various substrates. Preliminary investigation of animal preparations

(beef kidney, mouse liver and kidney) indicates no epimerizing activity for hydroxyproline.

617. METABOLISM OF ITACONIC ACID IN LIVER MITOCHONDRIA. Julius Adler (intr. by M. J. Johnson). Dept. of Biochemistry and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

Mitochondria from guinea pig liver, in the presence of Mg^{++}, completely oxidize itaconic acid (methylenesuccinic acid), but not mesaconic, citraconic, methylsuccinic, citramalic, itamalic, or β-methylmalic acids. Extracts of acetone-dried powders of guinea pig or rat liver mitochondria metabolize itaconate to mesaconate (methylfumarate), α-ketoglutarate, glutamate, lactate and CO_2, if ATP, Mg^{++}, and CoA are added. Mesaconate is not metabolized under these conditions. The initial step in the metabolism of itaconic acid appears to be its conversion to itaconyl CoA, which was detected by the formation of a hydroxamate; phosphorylating enzyme (succinate activating enzyme) catalyzes the formation of itaconyl CoA, but not mesaconyl CoA, if GTP and Mg^{++} are added. In the presence of itaconate, the extracts catalyze the fixation of CO_2 into citrate and the α-carboxyl carbon of glutamate. Similar extracts from biotin deficient rats catalyze this carboxylation less effectively. All data are consistent with the following sequence of reactions: itaconate $\xrightarrow{CO_2}$ tricarboxylic acid $\longrightarrow$ α-ketoglutarate $\longrightarrow$ glutamate. Since citrate or glutamate does not give rise to lactate in this system, the formation of lactate from itaconate indicates the existence of a second pathway for itaconate metabolism. Products were identified by chromatography in several systems, and in most cases by specific chemical degradations.

618. A DEXTRAN-HYDROLYZING ENZYME FROM INTESTINAL MUCOSA. George A. Adrouny,* Walter L. Bloom* and Alfred E. Wilhelmi. Dept. of Biochemistry, Div. of Basic Health Sciences, Emory Univ., Ga.

Extracts prepared from the mucosa of the small intestine of various mammalia - rat, guinea-pig, rabbit, pig, cow, dog and man - were found to induce an increase in the reducing power of dextran, a 1,6-glucosidic polysaccharide. Comparison of crude extracts from the various sources showed the preparation from the rat to be 60-80 times more active than those from the pig and the cow, while the activities of material from other species appeared to be at intermediate levels. Further experiments were therefore mostly carried out with extracts from the rat. Maximal activity was seen at pH 6.0-6.2. Preparations retained most of their activity for about 3 wk, if preserved below 4°C, at a pH of 6.0-7.0. Deterioration was very fast at a low pH, somewhat slower in alkaline media. The active principle is highly soluble in water; 50% saturated ammonium sulfate or 30-40% acetone, methanol or ethanol retain about 60% of the initial activity in solution. Other substrates that are hydrolyzed by crude or partially purified preparations include isomaltose, beta amylase limit dextrin, glycogen and starch; yeast mannan is not affected. Invertase accompanies "dextranase" in all fractions. Fractionation by organic solvents, adsorption on calcium phosphate, norit and diethylaminoethyl cellulose, precipitation with Zn^{++} and UO_2^{++}, and zone electrophoresis at various pH's were ineffective for the separation of the two principles. However, almost complete disappearance of invertase activity, with retention of about 90% "dextranase" activity, could be achieved by precipitation of the invertase with Hg^{++}.

619. BIOCHEMICAL CHANGES IN ARTICULAR CARTILAGE FOLLOWING DENERVATION OF THE EXTREMITY. Wayne H. Akeson* and Lillian Eichelberger. Orthopedic Surgery, Univ. of Chicago, Chicago, Ill.

Articular cartilage of puppies has been chemically examined to derive histochemical patterns of the cartilage. To estimate the effect of denervation of the extremity on cartilage, one hind leg of each puppy was denervated, the opposite leg being the weight bearing control leg. Histochemical comparisons were made: 1) on the consideration that 100 gm of cartilage solids are composed of a) an intracellular mass and b) an extracellular mass, represented by the combined weights of connective tissue solids and chondroitin sulfate. Thus, for one age group, in 100 gm of control cartilage solids the extracellular mass amounted to 71 gm of which 59 gm were connective tissue solids and 12 gm chondroitin sulfate, leaving 29 gm for cell solids. In 100 gm of cartilage solids from the denervated limb, the extracellular mass included 55 gm of connective tissue solids plus 11 gm chondroitin sulfate, leaving 34 gm for cell solids; 2) on the consideration that 1 kg of fresh cartilage is composed of an extra- and intracellular compartment. For control cartilage, the extracellular compartment totaled 722 gm of which 178 gm were solids and 544 gm, water; the intracellular compartment of 278 gm of which 68 gm were cell solids and 210 gm, cell water. For cartilage from the denervated extremity the extracellular compartment totaled 806 gm of which 141 gm were the solids and 665 gm, water, and the intracellular compartment of 194 gm of which 69 gm were the cell solids and 125 gm, cell water. (Aided by Grant A-972, Natl. Inst. of Arthritis and Metabolic Diseases, PHS.)

620. MICRO-DETERMINATION OF LYSINE IN PROTEIN HYDROLYSATES. Anthony A. Albanese and Louise A. Orto.* Nutritional Research Lab., St. Luke's Hosp., New York City.

In the light of recent developments, there now exists a need for accurate information on the lysine content of proteins occurring in human body tissues and foods. An extended evaluation of the available techniques led us to elaborate a chromatographic variant of the electrolytic method previously described by Albanese (J. Biol. Chem. 134:467, 1940). Briefly, the method involves the fractionation of hydrochloric acid hydrolysates of isolated proteins by 2 successive electrolyses to a catholyte containing the basic amino acids. The lysine in this fraction is determined chromatographically from recording densitometer readings at average Rf., 0.252 of unidimensional paper chromatograms prepared by our methods (J. Lab. & Clin. Med. 37:885, 1951). The analysis by the new and more rapid procedure requires 25-30 mg of total N. The percentage of lysine N of total N found for some protein products analyzed follows: lactalbumin (Sheffield) 9.30; casein (Borden vitamin-free) 8.30; skim milk protein (Borden's) 7.42; evaporated milk protein (Pet) 7.35; gelatin (U.S.P.) 6.12; human red blood cells (type B) 9.08; fibrin (Wilson) 8.60. These values are in good accord with those reported by others employing accurate macrochemical methods. Expression of amino acid N content in terms of total protein N provides a more biochemically useful basis of comparison than values calculated to an assumed 16.0% of N.

621. SERUM GLUTAMIC-OXALACETIC TRANSAMINASE FOLLOWING LOCAL INJURY. Harry G.

Albaum. Biology Research Dept., Brooklyn College, Brooklyn, N. Y.

Studies were undertaken to correlate biochemical changes in muscle after local injury with possible alterations in serum glutamic-oxalacetic acid transaminase activity. Standard procedures developed at the Randolph Air Force Base, Texas were employed to produce crush, tourniquet and radiation injury to the right hind leg of rabbits. Serum samples were assayed for glutamic-oxalacetic transaminase activity prior to and at varying intervals after injury. Radiation experiments were also carried out on the left chest wall in the area of the heart, and serum transaminase was measured before and after such treatment. It was found that crush, which within 24 hr causes a depletion of the ATP level of injured muscle, only produces a slight effect on serum transaminase, the maximum elevation occurring 1 hr after the onset of injury. Tourniquet, on the other hand, brings about an elevation in serum transaminase activity of almost 300%, the maximum alteration being attained 6 hr after the application of the tourniquet. By 48 hr the level has returned almost to normal. X-radiation of the right hind leg at dosages of 5000-25000 r brings about only a small rise in serum transaminase. This rise occurs only after 48 hr and by 8 days the level has returned to normal. Maximum change in muscle ATP in this instance starts at about the 5th day postirradiation. Local irradiation to the left chest wall brings about a significant increase in serum transaminase activity. The maximum effect is apparent at the end of 3 hr. Doses of 2500 r and above are effective. (Supported under Air Force Contract 18(600)543, USAF School of Aviation Medicine, Randolph Field, Texas.)

622. INTERFERENCE WITH ACYL TRANSFER BY A THIOCTIC ACID (TA) ANALOGUE, 6-ETHYL-8-MERCAPTOOCTANOIC ACID (6-EMO). Alberta M. Albrecht (intr. by Ernest L. Patterson). Nutrition and Physiology Section, Research Div., American Cyanamid Co., Pearl River, N. Y.

6-EMO is inhibitory for growth of certain microorganisms and its toxicity for Torula cremoris is reversed competitively by certain fatty acids including acetate, but not by (TA). The structural analogy between 6-EMO and 6-S-acetyl-6, 8-dimercaptooctanoic acid (6-acetyl-DMO), a functional form of TA, suggests that 6-EMO interferes with acyl transfer. The effect of 6-EMO on pyruvate oxidation by extracts of E. coli (fraction A) was studied. 6-EMO did not inhibit the anaerobic decarboxylation of pyruvate but inhibited pyruvate oxidation, thus demonstrating that 6-EMO interferes beyond the diphosphothiamine stage. Since these findings were consistent with the view that 6-EMO might interfere in that area of pyruvate oxidation mediated by TA, the synthesis of 6-acetyl-DMO (Gunsalus et al. J. Amer. Chem. Soc. 68:1763, 1956) was studied in the coupled enzyme reactions: 1) acetyl phosphate + CoASH → S-acetyl-CoA + iP; 2) S-acetyl CoA + 6, 8-dimercaptooctanoic acid → 6-acetyl-DMO + CoASH. Some evidence was obtained that 6-EMO blocked the formation of 6-acetyl-DMO, but the degree of inhibition varied with different enzyme preparations. However, 6-EMO interfered markedly with phosphotransacetylase activity when reaction 1) above was coupled with the arsenolysis reaction. This effect was reversed by DMO implying the possible involvement of DMO in transacetylase reactions.

623. THROMBIN FORMATION FROM PROTHROMBIN, PROCONVERTIN, AND CITRATE. Benjamin Alexander, Robert Goldstein* and Gabriel Izak.* Beth Israel Hosp. and Harvard Univ., Boston, Mass.

Thrombin elaboration from prothrombin in the presence of concentrated citrate has been explored with material of high specific activity obtained in our laboratory. Under these conditions thrombin does not form unless proconvertin-convertin or associated material is present as contaminant, or is provided as a highly purified fraction. The latter alone with citrate does not yield thrombin for 96 hr and maintains essentially full convertin activity. Moreover, during citrate incubation proconvertin-free prothrombin remains reactive to thromboplastin, Ac-globulin and calcium for at least 140 hr, whereas the proconvertin-rich prothrombin becomes less reactive as thrombin evolves. This apparent deterioration occurs within several hours, but can be substantially reversed when convertin-rich serum is added to the assay system, or when the citrate is removed by dialysis and the dialyzed fraction allowed to stand. Even after citrate removal some thrombin elaboration continues. The final total thrombin and assayable prothrombin was equivalent to the original prothrombin value. Immediately after citrate removal no "autoprothrombin" could be demonstrated by addition to fresh prothrombin. Not only was no accelerator activity found, but also full values for the additional prothrombin could not be demonstrated biologically in the combination. Thus, in citrate, as in the tissue thromboplastin system, prothrombin cannot be induced to yield thrombin unless convertin or something else in the convertin-rich fraction is also present. These observations do not support the view of other investigators that prothrombin contains all ingredients essential for thrombin formation. (Supported by the Office of the Surgeon General (DA-45-007-MD-171) and PHS H-656.)

624. CATALASE INHIBITION BY NORMAL AND NEOPLASTIC TISSUE EXTRACTS. Nicholas Alexander (intr. by Aaron B. Lerner). Radioisotope Service, VA Hosp., West Haven, and Dept. of Biochemistry, Yale Univ., New Haven, Conn.

When injected intraperitoneally, some alcohol precipitable fractions from neoplastic tissue extracts depress liver catalatic activity (H_2O_2 decomposition) but these same fractions do not inhibit catalatic activity in vitro. Other alcohol soluble fractions from boiled, aqueous, extracts of normal and neoplastic tissues (BAE) inhibit catalatic activity in vitro. Evidence is presented that BAE inhibition is due to substances which generate H_2O_2 on autoxidation. This generated H_2O_2 can be used by catalase to peroxidise ethanol to acetaldehyde, and ethanol completely reverses the catalatic inhibition by BAE. These results indicate that when catalase is exposed to BAE, some inactive catalase-H_2O_2 complex II (Biochem. J. 46:387, 1950) accumulates and ethanol removes and prevents the further accumulation of complex II. The Soret absorption spectra of catalase, catalase-BAE and catalase-BAE-ethanol mixtures support this. Ethanol causes the altered catalase-BAE spectrum to revert almost completely to that of free catalase. N-ethylmaleimide and ascorbic acid oxidase partially reverse BAE inhibition and most of the inhibition is due to the presence of sulfhydryl groups and ascorbic acid. Ethanol prevents in vitro catalatic inhibition by cysteine or reduced glutathione and is peroxidized to acetaldehyde in the process. Ethanol has no effect on the reduced catalatic activity of liver homogenates from rats injected with a catalase-depressing fraction obtained from a human carcinoma. It is concluded that the mechanisms of in vitro and in vivo catalase inhibition are not the same.

625. ENZYMES OF HISTIDINE BIOSYNTHESIS IN HISTIDINE-REQUIRING MUTANTS OF NEUROSPORA. Bruce N. Ames (intr. by James C. Reid). NIH, Bethesda, Md.

The pathway of histidine biosynthesis in Neurospora is: →Imidazoleglycerol phosphate —(IGP-dehydrase)→ Imidazoleacetol phosphate —(IAP-transaminase)→ L-Histidinol phosphate —(HP-phosphatase)→ L-Histidinol —(dehydrogenase)→ L-Histidine. Five genetically distinct histidine-requiring mutant types have been examined. These mutants appear to be lacking different enzymes in the biosynthesis, and characteristically accumulate large amounts of precursors before the blocked reaction. In a mutant the levels of enzymes before the blocked reaction may be considerably elevated. The enzymes after a blocked reaction appear to be present at the wild-type level; this level may be considered constitutive in that no substrate could have been present as an inducer. Two of the mutants are blocked before IGP and have about the same specific activity as wild type for IGP-dehydrase (Fed. Proc. 15:210, 1955), IAP-transaminase (J. Biol. Chem. 220:113, 1956) and HP-phosphatase (J. Biol. Chem. In press). The third mutant contains less than 1% of the IGP-dehydrase present in wild type and has normal amounts of the latter enzymes. The fourth mutant contains less than 2% of the normal amount of a specific hisitidinol phosphate phosphatase. Histidinol dehydrogenase (Adams. J. Biol. Chem. 209:829, 1954) is missing in the fifth mutant, which contains 3x the normal level of phosphatase. HP-phosphatase has been purified 12-fold from this mutant. The purified enzyme has negligible activity for IGP, IAP or ethanolamine phosphate and has a pH optimum of 9.0

626. IN VIVO ISOMERIZATION OF VITAMIN A ISOMERS. Stanley R. Ames, William J. Swanson* and Philip L. Harris. Research Labs. Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y.

Vitamin A-depleted albino rats were supplemented orally with 3000-7000 units of either all-trans-, 6-mono-cis-, or 2,6-di-cis-vitamin A acetate. Forty-eight hours after the final supplement the livers were removed and extracted. A portion of the liver lipids was analyzed for vitamin A by the Carr-Price reaction. The remaining lipids were then fed to additional groups of vitamin A-depleted rats and the extent of liver storage of vitamin A was determined. All-trans-vitamin A acetate was used as the reference standard. The relative biopotency of the vitamin A in the livers of rats fed all-trans-vitamin A acetate was only 64%. When the 6-mono-cis- and 2,6-di-cis-vitamin A acetates were fed initially, their relative biopotencies were 28% and 24%, respectively, corresponding to previously reported results (J. Am. Chem. Soc. 77:4134, 1955). The vitamin A in their livers gave relative biopotencies of 60% and 64%, respectively. Therefore, the rat isomerizes all-trans-vitamin A and stores a mixture of all-trans- and less active cis- isomers other than neovitamin A (2-mono-cis-). When the 6-cis- isomers with low biopotency are fed, they are isomerized in part to all-trans- vitamin A. Vitamin A appears to be stored in the rat liver as an equilibrium mixture of isomers independent of the isomer fed.

627. PURINE BIOSYNTHESIS IN AZASERINE-SENSITIVE AND -RESISTANT LINES OF THE MOUSE PLASMA CELL NEOPLASM 70429. E. P. Anderson, Bruce Levenberg and L. W. Law. (intr. by Walter C. Schneider). NIH, Bethesda, Md.

Specific enzymatic reactions in the de novo synthesis of purine mononucleotides have been investigated in various transfer lines of plasma cell neoplasm 70429. Lines sensitive and resistant to L-azaserine and cross-resistant to 6-diazo-5-oxo-L-norleucine (DON) have been developed in ascitic form in C3H mice (Potter and Law. J. Natl. Cancer Inst. 18:1957). It has been found that cell-free preparations of this mammalian tumor are able to carry out the following reactions: (α-N-formyl)-glycinamide ribotide (FGAR) $\xrightarrow[ATP]{\text{glutamine}}$ 5-aminoimidazole ribotide (AIR) $\xrightarrow[CO_2]{\text{aspartate}}$ 5-amino-4-imidazole carboxamide ribotide (AICR). These reactions have previously been characterized in pigeon liver (Levenberg and Buchanan. J. Am. Chem. Soc. 78:504, 1956; Lukens and Buchanan. Fed. Proc. 15:305, 1956). Synthesis of AIR and AICR has been measured by the Bratton-Marshall reaction. Extracts from both sensitive and resistant lines are qualitatively alike in their ability to catalyze the above reactions. Azaserine and DON inhibit the conversion of FGAR to AIR in both lines; the reaction is more sensitive to azaserine inhibition than in pigeon liver. Incorporation of glycine-1-C^{14} into acid-soluble purines by cell-free extracts is similarly inhibited by azaserine in both lines. However, with intact cells in vitro, uptake of glycine into purines has been observed to be inhibited by azaserine in the sensitive line but not in the resistant line, 70429-Az-R-1.

628. EFFECT OF FERRIC CHLORIDE AND CHOLIC ACID ON MOBILIZATION OF ELEVATED LIVER CHOLESTEROL IN THE MOUSE. W. L. Anthony,* W. T. Beher and G. D. Baker.* Edsel B. Ford Inst. for Med. Research, Detroit, Mich.

It has been reported that dietary ferric chloride is effective in preventing hypercholesterolemia in the cockerel. It has been suggested that this is due to the action of ferric ions upon cholic acid, resulting in precipitation of ferric cholate. Inasmuch as cholic acid is obligatory for cholesterol absorption, its removal resulted in reduced cholesterol levels. Cholic acid also prevents the mobilization of elevated liver cholesterol in the rat. Therefore, the removal of cholic acid by ferric ions should increase the rate of mobilization of accumulated liver cholesterol. Four groups of mice, with elevated liver cholesterol, were placed on a cholesterol-free diet, variously supplemented with cholic acid and ferric chloride. At weekly intervals, animals from each group were killed and liver total lipids, total cholesterol and phospholipid determined. From the analytical results, the following generalizations can be drawn: 1) the rate of cholesterol regression is not increased by dietary ferric chloride; 2) cholic acid does prevent mobilization of elevated liver cholesterol; 3) ferric ions do not prevent cholic acid absorption.

629. ELECTROPHORESIS OF BOVINE PLASMA ALBUMIN IN THE PRESENCE OF THIOCYANATE ION AND IN 2 M UREA. Koichiro Aoki* and Joseph F. Foster. Dept. of Chemistry, Purdue Univ., Lafayette, Ind.

It has been shown that bovine plasma albumin undergoes an isomerization reaction (N--F equilibrium) in the pH range 4.5-3.5 in chloride media (Aoki and Foster. J. Am. Chem. Soc. 78:3538, 1956). Below pH 3.5 the molecule expands. It has been suggested that the anomalous character of the titration curve of this protein above pH 3.5 can be explained on the basis of this equilibrium if it is assumed that the

carboxylate groups are stabilized in the N form. To test this idea further electrophoretic studies have now been conducted in the presence of 0.02 M thiocyanate ion, which is known to be much more strongly bound to this protein than is chloride. Two boundaries were found between pH 3.2 and 4.2, the composition shifting with pH in accord with an equilibrium which is third order in hydrogen ion. The entire curve is shifted downward in pH by 0.3 units relative to that in chloride (midpoint pH 3.7). Titration curves were conducted with thiocyanic acid at constant 0.02 M thiocyanate. The anomaly in the titration curve is shifted in almost exact accord with prediction based on the mechanism previously suggested. Hydrogen ion titration curves in the presence of 2M urea have previously been published (Foster and Sterman. J. Am. Chem. Soc. 78, 3656, 1956). It was shown that urea markedly enhances the hydrogen ion binding at a given pH and was suggested that this arises from a weakening of the intramolecular forces (probably hydrogen bonds) which stabilize the N form. On this basis it would be predicted that 2 M urea would shift the N--F transition to higher pH. Electrophoretic studies are in excellent accord with this prediction, the midpoint of the N--F equilibrium being shifted to pH 4.9. Calculations of molecular radii have been made using Henry's equation with results in essential agreement with previous findings.

630. ADENINE TRANSAMINASE. S. A. Armentrout* and E. Goldwasser. Dept. of Biochemistry and Argonne Cancer Research Hosp., Univ. of Chicago, Chicago, Ill.

T. Utilis when grown in the presence of adenine produces an enzyme, called adenase, which deaminates adenine. (Roush. Arch. Biochem. 50:510, 1954). The specific activity of the enzyme could be increased at least 100-fold by ammonium sulfate precipitation and protamine precipitation. When the active fraction was dialyzed for 12 hr it lost essentially all of its activity. Activity was restored by adding concentrated dialyzate. The cofactor activity of the dialyzate could be adsorbed on a column of Dowex-1 at pH 7.0 and eluted with dilute HCl. However, 2 fractions were required for full activity. Brewer's yeast extracts could also supply the cofactors. It was found, further, that the activity of the dialyzed enzyme could be fully restored by the addition of a mixture of pyridoxal phosphate, an α keto acid and Cu^{++} ions, while pyridoxal was ineffective. Chromatographic evidence indicated that the reaction was reversible, and that upon addition of large quantities of aspartic or glutamic acid and hypoxanthine to the active enzyme small quantities of adenine could be obtained. Other compounds tested as amino donors were 4,5,6-triamino pyrimidine, guanine, and 2,6-diamino purine. None were effective under the conditions of assay. It is proposed that the name adenase be reserved for a true deaminase and this enzyme be called adenine transaminase.

631. IDENTIFICATION OF A MAJOR URINARY METABOLITE OF NOREPINEPHRINE. Marvin D. Armstrong and Armand McMillan.* Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.

The finding that homovanillic acid is formed by methylation of homoprotocatechuic acid in the animal organism (Fed. Proc. 15:353, 1956) was suggestive that a similar reaction might occur with the 3,4-dihydroxy group of norepinephrine or of its metabolite 3,4-dihydroxymandelic acid, and that 3-methoxy-4-hydroxymandelic acid (VMA) might be excreted in the urine. One of the phenolic acids in urine (compound 10, J. Biol. Chem. 218:293, 1956) has chromatographic properties identical with those of synthetic VMA; most adults excrete about 1.5-3 mg VMA/mg creatinine, corresponding to about 2-4 mg/day. An increased amount of VMA was excreted by 2 patients given intravenous norepinephrine for support of blood pressure; 30% of the administered norepinephrine could be accounted for as extra VMA. Two patients with pheochromocytomas excreted 12 and 90 mg VMA/mg creatinine, respectively, preoperatively and 1.5 and 2.7 mg VMA/mg creatinine postoperatively. VMA was isolated from the urine of one of these patients; the isolated material corresponded with authentic optically active VMA in solubility, crystal form, m.p., rotation and chromatographic properties. The stability of VMA, the amount present and the ease of estimation, would appear to make its determination rather than that of catechol amines preferable for the detection of pheochromocytomas. Since it has been shown that radioactive norepinephrine and epinephrine give the same pattern of radioactive urinary metabolites (Schayer et. al., Am. J. Physiol. 182:285, 1955), it appears likely that both amines contribute to the VMA in urine but, under usual conditions, most of the VMA excreted probably arises from norepinephrine.

632. BASICITY OF PORPHYRINS. S. Aronoff. Inst. for Atomic Research and Dept. of Botany, Iowa State College, Ames.

Porphyrins in general form dibasic salts, but there is disagreement concerning the values of the dissociation constants (Neuberger and Scott. Proc. Roy. Soc. London 213A:307, 1952). In this study it is shown that dihydroporphines, as pheophytin a or $\alpha, \beta, \gamma, \delta$-tetraphenylchlorin, have distinct dissociation constants ($K_1 \sim 10^{-1}$, $K_2 \sim 10^{-5}$), as shown by spectrophotometric titration in acetonitrile with perchloric acid in dioxane. Porphines, as protoporphine or $\alpha, \beta, \gamma, \delta$-tetraphenylporphine, show no distinct monosalt, and equal dissociation constants are inferred ($K_1 = K_2 \sim 10^{-2}$). The nonexchangeability of the central hydrogens of pheophytin a in neutral solution (Weigl and Livingston. J. Am. Chem. Soc. 74:4160, 1952) appears to be a general property of porphyrins. In acidic solution, however, tetraphenylchlorin exchanges rapidly, probably instantaneously, with the tritium of tritium oxide. Half of the radioactivity of the disalt is lost on reconversion to the free base. This demonstrates the equivalence of the four central hydrogens of the disalt and permits radiometric titration of porphyrins.

633. ENZYMATIC DEGRADATION OF D-GALACTURONIC AND D-GLUCURONIC ACID. Gilbert Ashwell. NIH, Bethesda, Md.

An aqueous extract of rat liver acetone powder has been shown to be capable of metabolizing D-galacturonic acid in the presence of TPNH. The enzymatically active fraction has been partially purified (20-fold) by following the rate of oxidation of TPNH at 340 mμ. DPNH cannot substitute for TPNH in this reaction. D-glucuronic acid and D-glucuronolactone react at 65% and 20%, respectively, of the rate obtained with galacturonic acid. Glucose, galactose and uridinediphosphateglucuronic acid are not utilized as substrates. With TPNH generated by isocitrate and isocitrate dehydrogenase, all of the galacturonic acid disappeared to form a reaction product which was readily converted to L-xylulose by mild treatment with acid. Approximately 35% of the galacturonic acid metabolized was recovered as ketopentose. The

mechanism of the over-all reaction is not yet clear. However, in an analogous reaction, Mapson and Isherwood (Biochem. J. 64:13, 1956) have demonstrated the conversion of D-galacturonic methyl ester to L-galactonolactone in plants and it is known that this compound is being formed here. Since decarboxylation of the lactone would yield a product of lower oxidation level than xylulose, it is postulated that another intermediate compound, 3-keto-L-galactonic acid, is formed and that this compound is decarboxylated by mild acid hydrolysis. Preliminary experiments have shown that the enzyme preparation will catalyze the reduction of TPN with either L-galactonolactone or L-galactonic acid as substrate but is inactive towards DPN. Conditions under which the decarboxylation step may be demonstrated enzymatically are being investigated.

634. RNA TURNOVER AND DNA SYNTHESIS IN BACTERIOPHAGE-INFECTED BACTERIA. L. Astrachan* and Elliot Volkin. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

We have previously shown that RNA in bacteriophage-infected E. coli can incorporate and subsequently release $P^{32}O_4$ although the total amount of RNA remains constant. Shortly after P^{32} is presented to the infected bacteria, RNA incorporates more P^{32} than does DNA although DNA, as the only nucleic acid end product of the infected cells' metabolism, eventually incorporates far more P^{32} than does RNA. In order to investigate whether a relation exists between RNA turnover and DNA synthesis, conditions were chosen to control the amount of DNA synthesis. A few minutes after bacteriophage infection, the inhibitor chloramphenicol was added, followed by $P^{32}O_4$, the times of the two additions allowing incorporation of P^{32} into RNA but not DNA. After P^{32} incorporation into RNA had occurred, the culture was divided into two parts. To part a was added excess $P^{31}O_4$ to dilute out the $P^{32}O_4$, while part b was washed free of chloramphenicol before reincubation in excess $P^{31}O_4$. The presence of chloramphenicol in a inhibited P^{32} release from RNA and prevented DNA synthesis. The infected bacteria in b, however, released P^{32} from RNA and concomitantly incorporated P^{32} into newly synthesized DNA. These results, which indicate a relation between DNA synthesis and RNA turnover, have been qualitatively confirmed using adenosine-C^{14} as a nucleic acid precursor.

635. EFFECT OF SPECIFIC INHIBITORS OF ACID PHOSPHATASE ON OXIDATION OF KREBS CYCLE ACIDS BY RAT LIVER "MITOCHONDRIA." Bernard Axelrod and Grace Sewell Kilsheimer.* Dept. of Biochemistry, Purdue Univ., West Lafayette, Ind.

Applemans, Wattiaux and de Duve (Biochem. J. 59: 438, 1955) have presented evidence that the acid phosphatase occurring in conventional mitochondrial preparations is associated, in fact, with nonmitochondrial granules. Owing to the difficulty of effecting a good separation the possibility remains that some conventional mitochondria do contain acid phosphatase. Like these workers, we have found the phosphatase to be a latent enzyme released on aging the "mitochondria." We have further found that it resembles the human prostatic enzyme in being inhibited by L- but not by D-tartrate. We have therefore attempted to see if specific phosphatase inhibitors interfere with the oxidative activities of the mitochondria in order to a) elucidate the metabolic role of acid phosphatase in mitochondrial preparations and b) determine the probability of the coexistence of oxidative and acid phosphatase activity in "mitochondrial" granules. L-tartrate was tested on a "conventional" preparation of rat liver mitochondria, presumably containing the nonmitochondrial granules of de Duve and co-workers. No effect on Krebs cycle oxidation was noted. However, because of the possible difficulty of transport of the tartrate ion into the mitochondrion, the diethyl ester and the diamide of L-tartaric acid were also tested. (These two substances are inhibitors of the "prostatic type" phosphatase.) Both compounds, at concentrations of the order of 0.05 M significantly inhibited the oxidation of the following acids: citrate, ketoglutarate, succinate, malate and pyruvate.

636. METABOLISM OF THE O-PHOSPHODIESTER OF SERINE AND ETHANOLAMINE IN THE TURTLE. P. Ayengar* and Eugene Roberts. Dept. of Biochemistry, Med. Research Ins., City of Hope Med. Ctr., Duarte, Calif.

Experiments were performed in an effort to study the metabolic relationships of the O-phosphodiester of serine and ethanolamine (SEPD), a major component of the free or easily extractable amino acid fraction of tissues of turtles, alligators and snakes. Paper chromatographic procedures were employed for the separation of the DNP derivatives of ethanolaminephosphate (EP), serine phosphate and SEPD. After intracardial injection of P^{32} into turtles a rapid uptake of label took place into EP, phospholipids, phosphoproteins and a number of phosphorylated intermediates in the various tissues, but there was only a very slow appearance of P^{32} in SEPD. Radioactivity was first detected in SEPD in the kidney 48-72 hr after administration of P^{32}. No incorporation of P^{32} could be detected into SEPD in muscle, the tissue with the highest content of this substance. Work with different types of preparations which included isolated beating hearts, tissue slices, whole blood and isolated red cells and hemolysates, incubated under a variety of conditions, showed that only the intact nucleated erythrocytes exhibited a slight degree of incorporation of P^{32} into SEPD after prolonged incubation. Although the carbon from acetate-2-C^{14}, glycine-1,2-C^{14}, and uniformly labeled glucose was utilized metabolically by the erythrocytes, none of the label was found in SEPD. The data suggest that SEPD is probably not synthesized from small molecular weight precursors.

637. RESISTANCE OF METAL-ION COMPLEXES OF CONALBUMIN TO DIGESTION BY CHYMOTRYPSIN. Parviz R. Azari* and Robert E. Feeney. Dept. of Biochemistry and Nutrition, Univ. of Nebraska, Lincoln.

The action of chymotrypsin on crystalline conalbumin and its metal complexes was studied. This metal-binding protein from egg white strongly binds 2 atoms of iron and the iron complex has a red color. It has been found that, whereas conalbumin is rapidly digested by chymotrypsin, the iron complex of conalbumin is not digested, although the iron complex does not inhinit the enzyme. Conalbumin was rapidly digested as demonstrated by loss of chromogenic capacity (red color with iron) and hydrolysis to peptides. One-fourth and one-half of the chromogenic capacity was destroyed in 1 hr at pH 8.1 and 37°C with conalbumin-chymotrypsin weight ratios of 100 and 20, respectively. Iron conalbumin (2 atoms of iron) was not attacked even after 12 hr as shown by no fragmentation, no loss of chromogenic capacity, no change of electrophoretic mobility and crystallizability. The chymotrypsin was not inhibited as shown by experiments in which serum albumin, lysozyme or

conalbumin added to the iron-conalbumin-chymotrypsin mixture was rapidly attacked and in which serum albumin or lysozyme with similar levels of iron was rapidly attacked. Copper and zinc complexes were very slowly attacked, in agreement with their relative degrees of metal binding. Studies on various possible metal-conalbumin complexes will be discussed. The resistance of the metal complexes might be interpreted as due to changes in molecular shape or charge distribution caused by complex formation, as well as to the more obvious possibility of the binding of the susceptible sites by iron.

638. INCORPORATION OF ACETATE-1-C^{14} INTO CHOLESTEROL BY HUMAN BRAIN TUMORS. Daniel L. Azarnoff,* George L. Curran and William P. Williamson.* Depts. of Internal Medicine and Surgery, Univ. of Kansas Med. Ctr., Kansas City.

Several investigators have demonstrated that adult mammalian central nervous system tissue does not appreciably incorporate C^{14}-labeled acetate into cholesterol in vivo or in vitro. Adult normal brain and tumor was obtained by sharp dissection at the time of craniotomy and immediately placed in cold buffer. Tissue slices were prepared and incubated with sodium acetate-1-C^{14} for 2 hr. At the end of the incubation period cholesterol was isolated as the digitonide, purified as the dibromide and its radioactivity determined with an end window Geiger-Müller tube. Normal white matter exhibited no C^{14} incorporation into cholesterol whereas the tumors gave specific activities ranging from 23,000 to 200 with the following relationship: ependynoma > glioblastoma > multiforme > meningioma > astrocytoma > normal brain. The total cholesterol concentration was 124-143 mg/gm dry tissue for normal brain and 17-93 mg/gm dry tissue for the tumors with an increase in the amount of esterified cholesterol. For comparison, the specific activity of cholesterol from an adenocarcinoma of the sigmoid colon was much below that of adjacent normal mucosa and the tumor's cholesterol concentration greater than the normal mucosa. This synthetic process in brain malignancies may afford an opportunity to study, in man, compounds such as vanadium which inhibit the synthesis of cholesterol. (Supported in part by grants from the Kansas Heart Assn. and #H-1947(C-2) from the Natl. Insts. of Health, PHS.)

639. LIPASE ASSAYS. Arthur L. Babson and Sylvia Malament (intr. by George H. Mangun). Warner-Chilcott Research Labs., Morris Plains, N. J.

The primary objections to the Cherry and Crandall lipase assay are the long incubation time (24 hr) and the instability of the olive oil emulsion substrate. Boissonnas (Helvet. chim. acta 31:1571, 1948) introduced a lipase assay using Tween 20, a water-soluble ester of lauric acid, as substrate in a 10-min assay. The Boissonnas assay was modified in the following ways: The control was a titration of the reaction mixture at zero time rather than the sum of the separate titrations of enzyme solution and substrate, since the Tween raised the pH of the end point. A 1-hr incubation gave better titrations than a 10-min incubation, and aurin gave a sharper end point than phenol red. Comparison of the two assay methods revealed that they were not measuring the same kind of activity. The Boissonnas assay is relatively insensitive to pancreatin and commercial lipase. When the lipase activity is plotted against the concentration of pancreatin a straight line on rectilinear coordinates is obtained with the Boissonnas assay but not with the Cherry and Crandall assay. The latter assay gives a straight line on semilogarithmic coordinates. The Boissonnas method of lipase purification from pancreatin gave a 10-fold increase in specific activity as measured by the Boissonnas assay and a 4-fold decrease in specific activity by the Cherry and Crandall assay. The data emphasize that the assay method is critical in evaluating lipase activity.

640. HYDRAZINE AND NITROGEN FIXATION. Michael K. Bach* and R. H. Burris. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Hydrazine at concentrations of 10^{-4} to 10^{-5} M disappeared from dense suspensions of Azotobacter vinelandii cells in phosphate buffer at pH 6.8. The disappearance of hydrazine was caused only by living cells, was stimulated by the addition of potassium cyanide and acetaldehyde and was inhibited by ammonium sulfate. Oxidation by preformed hydrogen peroxide in the cell suspensions could account for no more than 1% of the hydrazine which disappeared. When hydrazine labeled with N^{15} was supplied to the cells, part of the N^{15} was recovered in $N^{15}H_4^+$ and $N_2{}^{15}$. Between 60 and 90% of the hydrazine which disappeared could be recovered as dihydropyridazinone-5-carboxylic acid (PCA) and two other products which are formed in the chemical reaction between α-ketoglutarate and hydrazine. The conditions for the chemical formation of PCA, as well as some of its properties were studied. By the application of ion exchange and paper chromatography, PCA was shown to occur in A. vinelandii cells as well as in soybean nodules. Preliminary evidence from experiments with $N_2{}^{15}$ suggested that PCA or a closely related compound could serve as an intermediate in biological nitrogen fixation. The concentration of N^{15} in PCA frequently was equal to or higher than that in glutamic acid and ammonia from the same soybean nodules, and PCA had a much higher N^{15} concentration in A. vinelandii cells supplied with $N_2{}^{15}$ than in those supplied $N^{15}H_4^+$.

641. ROLE OF HYDROXYLAMINE IN THE CARBON DIOXIDE-ACTIVATING ENZYME SYSTEM. B. K. Bachhawat,* F. P. Kupiecki* and M. J. Coon. Dept. of Biological Chemistry, Med. School, Univ. of Michigan, Ann Arbor.

Earlier studies in this laboratory led to the demonstration that an activated form of carbon dioxide, believed to be adenosine phosphoryl carbonate (AMP-CO_2), is involved in the following reaction sequence: 1) CO_2 + ATP ⟶ AMP-CO_2 + pyrophosphate; and 2) AMP-CO_2 + β-hydroxyisovaleryl CoA (HIV CoA) ⟶ AMP + β-hydroxy-β-methylglutaryl CoA (HMG CoA). Pig heart extracts which catalyze these reactions contain an enzyme ("F enzyme") which brings about the fluoride-requiring, bicarbonate-dependent cleavage of ATP to ADP and fluorophosphate, as shown by Flavin, Castro-Mendoza and Ochoa. It has recently been found in this laboratory, as would be predicted from the products of reaction 1, that F enzyme plays no role in carbon dioxide activation for the carboxylation of HIV CoA. Reaction 1 has been found to be catalyzed by an enzyme ("H enzyme") which effects the hydroxylamine-requiring, bicarbonate-dependent cleavage of ATP to AMP and pyrophosphate, presumably by the following mechanism: 3) AMP-CO_2 + NH_2OH ⟶ AMP + carbonohydroxamic acid. H and F enzymes have been partially purified and completely separated from each other and from HIV carboxylase, which catalyzes reaction 2. Reaction 1 proceeds to the right only when coupled with reaction 2 or reaction 3; the AMP

was formed has been identified chromatographically and by the action of adenylic deaminase. Attempts to detect carbonohydroxamate have so far proved unsuccessful, but ethyl carbonohydroxamate has been identified chromatographically as the product of the action of hydroxylamine on the synthetically prepared ester, AMP-CO_2-C_2H_5.

642. PRODUCTION OF HYDROCORTISONE BY A HUMAN TESTICULAR TUMOR. Billy Baggett,* Lewis L. Engel, Linda L. Fielding,* Kenneth Savard, Ralph I. Dorfman, Frank L. Engel and Harry T. McPherson.* Huntington Memorial Labs. and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.; Worcester Fndn. for Expl. Biology, Shrewsbury, Mass.; and Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.

The production of steroids by a large undifferentiated testicular tumor has been studied. The patient, aged 3, had high urinary 17-ketosteroids and corticoids prior to removal of the tumor. Slight obesity and hypertension were the only signs of hormonal imbalance manifest in the patient. Paper chromatograms of the urinary steroids revealed elevated levels of 11 β-hydroxyandrosterone, dehydroepiandrosterone, etiocholanolone, tetrahydrocortisone and tetrahydrocortisol. Slices of the tumor were incubated with 540 μc of $CH_3C^{14}OONa$. Ten milligrams each of a number of steroids were added to the incubation mixture and an extract was prepared. Paper chromatograms of aliquots of this extract were radioautographed. Areas of darkening corresponding to the hydrocortisone, 11 β-hydroxy-4-androstene-3, 17-dione, 4-androstene-3,17-dione and dehydroepiandrosterone areas were observed. The hydrocortisone was isolated from the extract by chromatography on silica gel and a countercurrent distribution. In the distribution a peak in radioactivity coincided with the hydrocortisone peak as measured by ultraviolet absorption. Additional hydrocortisone carrier was added to the isolated material prior to crystallization to constant specific activity. A portion of the hydrocortisone was oxidized with chromic acid to adrenosterone, which on isolation by countercurrent distribution had a specific activity corresponding with that expected from the oxidation of biologically labeled hydrocortisone. Calculations based on reverse isotopic dilution showed that 511,000 cpm were incorporated into hydrocortisone from the labeled acetate.

643. DESTRUCTION OF ASCITES TUMOR NUCLEI BY HEPARIN. Nome Baker, Manuel Tubis, and Edward D. Hudack (intr. by Sidney Roberts). VA Center, Dept. of Physiological Chemistry, Univ. of California Med. Center, Los Angeles, and Radioisotope Service, VA Hosp., Cleveland, Ohio.

Roberts and Anderson (Exp. Cell Res. 2:24, 1951) and Anderson and Wilbur (J. Gen. Physiol. 34:647, 1951) have reported that heparin causes the release of DNA and histone from cell nuclei of homogenized liver. It was suggested by these workers that this phenomenon proceeded unaccompanied by a gross breakdown of the nuclear membrane. However, it has been observed in this laboratory that similar concentrations of heparin (1-2 mg heparin, 2-8 x 10^6 nuclei per cc buffer) cause an almost instantaneous destruction of from 50 to 80% of nuclei from the Ehrlich ascites tumor at either 0° or 25°C. At these concentrations of heparin the destruction seems independent of the presence of cytoplasm. Since a rapid disappearance of normal liver nuclei following heparin treatment apparently has not been observed by other workers, several of the experiments of Anderson's group were repeated using normal liver nuclei. The latter were destroyed at a rate similar to that of tumor nuclei. By using concentrations of heparin as low as 16 μg/cc it was possible to demonstrate that, during the reaction with cancer nuclei, heparin becomes "bound;" it is then able to exert only a small fraction of its original effect when fresh nuclei are added to the reaction mixture. Measurable rates of destruction have been observed using as little as 0.17 μg heparin/cc. Studies with P^{32}-labeled nuclei have demonstrated a wide variation in the capacity of isolated cancer nuclei to resist heparin destruction.

644. HISTIDINE METABOLISM IN VITAMIN B_6- AND IN BIOTIN-DEFICIENT RATS. Robert C. Baldridge and Charles D. Tourtellotte (intr. by Howard W. Robinson). Dept. of Physiological Chemistry, Temple Univ. School of Medicine, Philadelphia, Pa.

A major route in the metabolism of histidine is via straight deamination. Biotin has been implicated as a cofactor in an analogous reaction in bacteria (conversion of aspartate to fumarate). Because of this and the multiple metabolic role of pyridoxine derivatives, studies were made of the effects of lack of these vitamins on liver enzyme systems involved in straight deamination of histidine. Levels of histidase and urocanase were measured by a spectrophotometric procedure (J. Biol. Chem. 201:775, 1953). Aspartic-glutamic transaminase and rhodanese concentrations were also determined; the former as an indication of depletion of tissue pyridoxal phosphate and the latter as an index of effects of vitamin deficiencies on enzyme protein synthesis. Elevated levels of histidase in the livers of vitamin B_6-deficient rats (difference from pair-fed control rats was highly significant, $P < 0.01$), accompanied depressed transaminase activity. Urocanase and rhodanese levels were not significantly different from control values. Increased levels of liver histidase were also observed in biotin-deficient animals. (Difference from pair-fed controls was significant, $0.01 < P < 0.05$). Urocanase, rhodanese and aspartic-glutamic transaminase were essentially unchanged. In addition to straight deamination, histidine undergoes transamination for which pyridoxal phosphate is required. Results with pyridoxine-deficient animals can be interpreted as an adaptive increase in the enzyme which catalyses straight deamination (histidase) in response to denial of the route involving transamination. (Supported by Grant A650, Natl. Insts. of Health, PHS.)

645. UTILIZATION OF 6-MERCAPTOPURINE BY STREPTOCOCCUS FAECALIS. M. Earl Balis and Dorris J. Hutchison.* Sloan-Kettering Div., Cornell Univ. Med. College, New York City.

Investigations into the effects of 6-mercaptopurine on the utilization of various purines and purine derivatives by a series of drug resistant mutants of Streptococcus faecalis led to the observation that certain mutants appeared to be able to use this antagonist as a purine source. Those purines which S. faecalis can incorporate into nucleic acid reverse the toxicity of 6-mercaptopurine. The fact that certain strains which were resistant to the antimetabolite appeared to use it as a purine source implied that perhaps this was one mechanism by which resistance developed. When the bacteria were grown with labeled purines the presence of unlabeled 6-mercaptopurine led to a reduction of the amount of label found in the nucleic acid of the bacteria. The growth of the bacteria in the presence of 6-mercaptopurine-8-C^{14} led to the

presence of nucleic acid adenine and guanine containing C^{14} at a level comparable to that obtained with hypoxanthine-8-C^{14}. The incorporation was probably not due to chemical hydrolysis of the mercaptopurine since even small amounts of hypoxanthine are detectable spectrally in solution of mercaptopurine and none was so detected in the medium. Work with Lactobacillus casei (Balis et al.) has led to the conclusion that in exerting its action 6-mercaptopurine is first converted to a ribosyl derivative, the data with S. faecalis would imply that this compound could be converted to an inosine derivative which serves as a source of nucleic acid purines.

646. STUDIES ON ENOLASE. Clinton E. Ballou and Finn Wold.* Dept. of Biochemistry, Univ. of California, Berkeley.

The equilibrium of the enolase reaction is affected by both pH and the metal ion concentration. The variations of the apparent equilibrium constant can be theoretically related to the difference in the ionization constants of the two substrates and to the differences between their affinities for the activating metal. When the ionization constants and the metal binding constants were determined by titration of the substrates, the metal and pH independent equilibrium constant could be calculated and was found to be 6.3. Studies of the kinetics of the enolase reaction have clarified the mechanism of the substrate inhibition and have given some data relating to the structure of the active site of the enzyme. Thus the effect of several substrate analogs has indicated that a compound must contain both a carboxyl and a phosphate group in order to combine with the active site. pH studies have shown that only the totally ionized substrates are being acted upon by the enzyme, and also that the active site of enolase appears to have an ionizable group with a pKa of about 7.5. Certain data pertaining to the role of the activating metals will also be presented.

647. LIVER LACTIC DEHYDROGENASE—FURTHER INHIBITION STUDIES. James N. Baptist* and Carl S. Vestling. Dept. of Chemistry and Chemical Engineering, Univ. of Illinois, Urbana.

Extensive inhibitor studies with pure rat liver lactic dehydrogenase (LDH) have provided the opportunity of analyzing the inhibition as a function of the chemical structure of the inhibitors and of pH, the reactions being measured from both the lactate-DPN^+ and pyruvate-$DPNH\text{-}H^+$ sides of the equilibrium. Data from experiments with benzene sulfonate, p-chlorobenzene sulfonate, p-toluene sulfonate, oxalate, o-phthalate and an o-phthalate-ethylene glycol ester analyzing for two phthalate residues and one glycol residue have been subjected to study. Benzene sulfonate inhibition fits a formulation where inhibitor reacts competitively at the lactate site with either free LDH or with LDH·DPN^+ (case 1). The p-substituted benzene sulfonates fit the situation where inhibitor combines competitively at the lactate site but can be displaced by either lactate or DPN^+, apparently because of the steric influence of the p-Cl or p-CH_3 group (case 2). Oxalate, however, fits the case where inhibitor reacts at the lactate site but only with LDH-DPN^+ and not with free LDH (case 3). Since p-chlorobenzene sulfonate presumably combines at the lactate (or pyruvate) site on LDH, the same inhibitor constant should obtain when measured from either side of the equilibrium. For such a calculation one requires all the kinetic constants for the uninhibited system. These have been determined, and a value of 6 to 8×10^{-3} M has been obtained in this way. Inhibition from the pyruvate side by p-chlorobenzene sulfonate is a function of pH with strong inhibition at pH 9 and no inhibition at pH 6.5, which is close to the optimal pH. This result suggests the possibility of determining the pK'a of a group on or near the active site of LDH.

648. TRANSAMINATION REACTIONS IN MAMMALIAN TISSUE CULTURE CELLS. S. Barban (intr. by M. Silverman). Natl. Insts. of Health, Bethesda, Md.

A comparative study on the scope and pattern of transamination reactions in normal and malignant cells in tissue culture was undertaken. Employing intact cells and cell-free extracts prepared by sonic disruption it has been found that both the mouse fibroblast (strain L) and a human uterine carcinoma cell (strain HeLa) can synthesize most of the nonessential amino acids through transamination reactions. Some notable quantitative differences in the metabolic pattern of the cell lines have been observed. Certain amino acids, particularly glutamic aspartic, isoleucine, leucine and alanine, were most active as amino donors. Other amino acids tested were relatively weak or inert. Transamination reactions involving glutamine, an essential metabolite of these cells, have been demonstrated. Glutamic acid was, however, more effective than glutamine as an amino donor in various transaminase systems and was indeed the most active of the amino donors tested.

649. INHIBITORS OF DE NOVO FORMYL GLYCINAMIDE RIBOTIDE SYNTHESIS IN PIGEON LIVER EXTRACTS. W. Barg,* E. Boggiano,* N. Sloane and E. C. De Renzo.* Research Div., American Cyanamid Co., Pearl River, N. Y.

A norite-treated soluble pigeon liver enzyme system was found to synthesize formyl glycinamide ribotide (FGAR) from glycine, glutamine, adenosine triphosphate, citrovorum factor (CF), ribose-5-phosphate and a one carbon donor. No requirement for copper ions could be demonstrated. The enzyme system was prepared by centrifugation of a pigeon liver homogenate at 35,000 x g. The supernatant fraction was separated into 48% (V/V) acetone soluble and insoluble fractions. Both fractions were required for FGAR synthesis. The 48% acetone soluble fraction appeared to enzymatically activate CF to N^{-10}-formyl tetrahydrofolic acid or a closely related compound; anhydroleucovorin or isoleucovorin could replace CF + activating enzyme. FGAR formation was determined by the addition of inosinic acid (IMP) as one carbon donor according to the following equation (Warren and Flaks. Fed. Proc. 15:379, 1956): GAR + IMP $\longrightarrow$ FGAR + AICR. Under these conditions the formation of 5-amino-4-imidazolecarboxamide ribotide (AICR) was quantitatively measured as nonacetylated arylamine. From a variety of compounds studied (1-2 μ moles/ml) only the following were found to inhibit the above system: the diazo antibiotics, 6-diazo-5-oxo-1-norleucine and alazopeptin (azaserine to a much lesser extent); histidine metabolic products, e.g., formimino-glutamic acid > urocanic acid >> histidine; promazine and chloropromazine (chloropromazine sulfoxide was inactive); pyridoxal phosphate. The inhibitory action of the compounds mentioned above was found to be reversed by higher levels of glutamine or by the addition of preformed glycinamide ribotide (GAR). The inhibitory activity of the following compounds was not reversed by higher levels of glutamine or by the addition of preformed GAR: D-lysergic acid diethylamide tartrate and triphospho-pyridine nucleotide (TPN). Lower levels of TPN were sometimes slightly stimulatory.

650. GLUTAMATE DECOMPOSITION AND SYNTHESIS VIA MESACONATE BY EXTRACTS OF CLOSTRIDIUM TETANOMORPHUM. H. A. Barker, R. Marilyn Wilson* and Agnete Munch-Petersen.* Dept. of Agricultural Biochemistry, Univ. of California, Berkeley.

Glutamate decomposition by extracts of C. tetanomorphum was shown previously to involve the following reactions: 1) glutamate ⟶ mesaconate + NH_3; 2) mesaconate + H_2O ⟶ d-citramalate; and 3) d-citramalate ⟶ pyruvate + acetate. All of these reactions are readily reversible under appropriate conditions; thus they provide a pathway for glutamate synthesis from acetate, pyruvate and ammonia. Reaction 2 requires cysteine and ferrous ion as cofactors. Consequently reaction 1 can be separated from other reactions in unfractionated extracts by the addition of reagents that bind ferrous ion. Reaction 1 involves the interconversion of straight and branched chain 5-carbon compounds. The terminal methyl group of mesaconate is derived from carbon atom 3 of glutamate. This indicates that formation of the branched chain results from cleavage of the bond between carbon atoms 2 and 3 of glutamate followed by formation of a new bond between carbon atoms 2 and 4. Both D- and L-glutamate are utilized and formed in this system. The participation of cofactors and possible intermediates in reaction 1 will be discussed.

651. FORMATION OF METHYLMALONATE IN RAT LIVER. Lewis Barness,* Ottavio Barnabei,* Aree Valyasevi* and Paul György. Dept. of Pediatrics, School of Medicine, Univ. of Pennsylvania, Philadelphia.

Rats fed a yeast-containing diet which produces liver necrosis excrete methylmalonic acid in their urine. Excretion of this acid is decreased by the oral administration of Aureomycin or Penicillin (J. Biol. Chem. 221:93, 1956). Using animal enzyme systems, Coon (Fed. Proc. 14:762, 1955) indicated the formation of methylmalonate from valine. Using dialyzed extracts of pig heart, Flavin (Nature 176:823, 1955) condensed CO_2 with propionyl CoA to form methylmalonate. Similarly, Katz and Chaikoff (J. Am. Chem. Soc. 77:2659, 1955), using rat liver slices, formed methylmalonate from CO_2 and propionyl CoA. Livers of adult male rats were perfused (Miller. J. Exper. Med. 94:431, 1951) with a mixture of blood, saline, sodium malonate and the test substance. The infusion mixture was recirculated, acidified and extracted with ether. Methylmalonic acid was identified by paper chromatography and, after elution, by mixed melting point. Methylmalonic acid was found in the perfusion mixture when l-valine or when sodium propionate was added to the infusion. Aureomycin added to the mixture did not alter the concentrations of methylmalonate produced. These data support the hypothesis that methylmalonate (or its CoA derivative) is a reaction product in the metabolism of valine and in the carboxylation of propionic acid in the living liver. The action of aureomycin in decreasing the urinary excretion of methylmalonate in the intact animal is apparently not a direct metabolic effect.

652. STRUCTURAL ORGANIZATION OF PHOSPHORUS TRANSFERRING REACTIONS OF THE RED CELL. Grant R. Bartlett. Scripps Clinic, La Jolla, Calif.

The normal red cell surface is impermeable to inorganic phosphate (iP) from inside or out. P^{32}, added to the medium as iP, appears in the cellular glycolytic intermediates through some reaction at the surface and at a rate indicating that only external iP is utilized. During steady state glycolysis: glucose + 2 extracellular iP⟶2 lactate + 2 iP. No product iP accumulates and it must be transferred out of the cell as formed. One can either resort to unknown two-way transport devices or put some of the phosphorus transferases in the surface. Glyceraldehyde dehydrogenase would have to bring together iP from the outside, glyceraldehyde phosphate from within and release product 1,3-DPG inside. Because of the tight binding of DPN-DPNH to glyceraldehyde dehydrogenase and lactic dehydrogenase it would be convenient to couple these two in the surface. Since product iP must be removed from the cell it would be logical to have ATPase in the surface, using the energy released for any work needs. This enzyme then must be available to the cellular ATP, put iP out and ADP in. There is evidence that glucose is used only from the outside, requiring hexokinase in the surface. The lack of cellular triosephosphate and 1,3-DPG could be explained by a TP isomerase-glyceraldehyde phosphate dehydrogenase-phosphoglycerokinase chain, the first and the last adjacent to the inside surface, supplying substrate and removing product for the glyceraldehyde dehydrogenase reaction. The two mutases and the hexose phosphate isomerase and kinase could be freely dispersed in the cytoplasm. The amounts of intermediates and P^{32} SA data will be discussed on the hypothesis that glycolysis operates as a structurally organized pattern of enzymes rather than in a homogeneous phase.

653. SHORT-TERM EFFECTS OF GROWTH HORMONE ON OXIDIZED AND REDUCED LIVER COENZYME A IN THE RAT. Paul Bartlett and Pamela Grimmett.* Edsel B. Ford Inst. for Med. Research, Detroit, Mich.

We have previously reported an increase in liver coenzyme A concentrations in normal adult rats killed upon termination of a 10-day period of stimulation with daily doses of 0.5 mg of growth hormone (J. Biol. Chem. 218:419, 1956). The present studies, also on normal adult rats, concern the relatively short-term effects of single 0.5-mg doses of growth hormone on concentrations of total, oxidized and reduced liver coenzyme A, as determined by the method of Boxer and Shonk (Arch. Biochem. 59:24, 1955). In animals killed at 5- or 16-hr intervals after stimulation with the hormone, liver concentrations of reduced coenzyme A, expressed in terms of Kaplan-Lipmann units/100 gm body weight, were found to be significantly higher ($P < 0.01$) than those in untreated weight controls. Although concentrations of oxidized coenzyme A were substantially reduced in livers of rats killed at both the 5- and 16-hr time intervals, these differences were significant only at P values of 0.02 and 0.05, respectively. In rats killed 5 hr after hormonal stimulation, total liver coenzyme A concentrations were significantly higher ($P < 0.01$) than in normal weight controls. Total liver coenzyme A concentrations of animals killed 16 hr after treatment were not significantly different from those of the untreated weight controls. In rats killed at 5- and 16-hr intervals after treatment with growth hormone, ratios of oxidized to reduced liver coenzyme A were found to be significantly lower than in normal weight controls ($P < 0.01$). Results of similar studies on hypophysectomized rats will also be presented.

654. FRAGMENTATION OF THE SUCCINIC DEHYDROGENASE COMPLEX (SDC). R. E. Basford,* J. Jarnefelt,* H. Tisdale* and D. E. Green. Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

SDC is a particulate entity obtained by fragmentation

of beef heart mitochondria which contains succinic and DPNH heme chains in a ratio of 3 : 1 (J. Biol. Chem. 217:551, 1955; Biochim. et biophys. acta, in press). Each of the two respective heme chains of KC1 fractionated SDC contains a specific flavoprotein, cytochrome b and cytochrome c_1 in a molecular ratio of 1 : 1 : 1. The two particulate heme chains can be separated from one another by subjecting SDC to fractionation with cholate and ammonium sulfate. By treatment of the two particles with lipophilic reagents each may be fragmented and separated into functionally and spectroscopically pure ultimate components, viz, the soluble dehydrogenase, cytochromes b and c_1 in particulate form and soluble lipoproteins. The composition and properties of these ultimate units of the electron transfer system will be discussed.

655. MECHANISM OF CONVERSION OF PLASMA PROTEOLYTIC ENZYME PRECURSOR TO THE ACTIVE ENZYME. Werner Baumgarten, Rose B. Cole and Maurel N. Richard (intr. by L. Earle Arnow). Merck Sharp and Dohme Research Labs. Div. of Merck & Co., Inc., West Point, Pa.

The euglobulin fraction of human and bovine plasma contains a proteolytic enzyme precursor. The conversion of this precursor to the active enzyme by the human activator is compared to its activation by chloroform or glycerol. The course of the conversion and the total yield of proteolytic enzyme is followed by fibrin clot dissolution and by casein hydrolysis. The formation of proteolytic enzyme is demonstrated to be an enzymatic reaction and is independent of the concentration of human activator present, while the reaction between streptokinase and the human euglobulin fraction is stoichiometric. The evaluation of the reaction between human activator and human euglobulin fraction depends upon the method of analysis. The apparent yield of proteolytic enzyme is greater if casein hydrolysis rather than clot lysis is employed. These results may be explained by streptokinase inhibition of casein hydrolysis. Free streptokinase present in the activator is diminished in contact with unreacted proactivator. This occurs when human euglobulin is added to human activator. Fibrin lysis is not inhibited by excess of streptokinase.

656. LIPOGENESIS IN ADIPOSE TISSUE OF TUMOR-BEARING RATS. R. W. Begg and J. A. Trew.* Collip Med. Research Lab., Univ. of Western Ontario, London, Canada.

The loss of fat from the depots of tumor-bearing rats has been attributed to mobilization of lipid for energy purposes. We have been unable to deplete nontumor rats of lipid to the same extent as tumor-bearing rats, by caloric restriction (Cancer Res. 16: 548, 1956) and have considered the possibility of a defective lipogenesis interfering with replacement of depot fat in the tumor-bearing rat. Adipose tissue was minced with scissors and incubated under oxygen in a buffer containing acetate-1-C^{14} or universally labeled glucose. Respiratory CO_2 was collected and fatty acids extracted after saponification of the tissue. Incorporation of labeled acetate or glucose into the fatty acids of adipose tissue from tumor-bearing rats is much less than that of control rats. Tube-feeding of glucose before the rats were killed, or addition of glucose to the medium, increases the incorporation of acetate in both groups, but the tissue from tumor-bearing rats still exhibits reduced incorporation of acetate when compared with the controls. Pair-feeding experiments suggest that the results are not due to reduced food intake by the tumor-bearing rats, and the counts in respiratory CO_2 indicate an adequate activation of acetate.

657. EFFECTS OF FLAVONOIDS ON INFLAMMATION. J. M. Beiler,* R. R. Brendel* and G. J. Martin. Research Labs. Natl. Drug Co., Philadelphia, Pa.

Certain of the flavonoid compounds have been reported to have anti-inflammatory action. This action has been explained as a function of their effects on the vascular system. In order to investigate this phenomenon, the effects of flavonoids in experimental inflammations of varying etiology and severity were assayed. Inflammation was produced by cold injury, local egg-white injection and subcutaneous pellet implantation. Among the flavonoids tested were rutin, hesperidin, catechin and a number of hydroxylated chalcones. Ascorbic acid was also studied. Anti-inflammatory effects were demonstrated under the experimental conditions utilized. A comparison of the effects of the flavonoids with those of trypsin and cortisone showed dissimilarities, indicating differences in mechanism of action.

658. CHEMICAL SYNTHESIS AND ENZYMATIC UTILIZATION OF ADENYL AMINO ACIDS. Paul Berg. Dept. of Microbiology, Washington Univ. School of Medicine, St. Louis, Mo.

Amino acid acyl phosphate derivatives of adenosine-5'-P, which are presumably formed from ATP and amino acids, have been suggested as intermediates in the de novo synthesis of proteins. To study this question a chemical method for the preparation of such adenyl amino acids has been developed. One equivalent each of amino acid, adenosine-5'-P, HCl, and 24 of dicyclohexyl carbodiimide are incubated for 3-5 hr in 14% aqueous pyridine at 0°. The adenyl amino acid is obtained in 40-50% yield by precipitation with 15 volumes of cold acetone. The purity is 50-65%, based on the adenosine-5'-P content. Adenyl-L-methionine was further purified by paper ionophoresis at pH 3.2 and gave the following ratios for adenosine-5'-P: adenine : total methionine : acyl bound methionine; 1.0 : 1.02 : 1.10 : 0.91. Of 14 amino acids tested only glutamic, aspartic and histidine failed to yield detectable amounts of the adenyl derivatives. Synthetic adenyl-L-methionine is rapidly converted to ATP in the presence of inorganic pyrophosphate (PP) and a partially purified enzyme from yeast or with dialyzed extracts of E. coli according to the following reaction: 1) adenyl-methionine + PP $\rightleftharpoons$ ATP + methionine. Thus in 5, 10 and 15 min there was a disappearance of 0.12, 0.23 and 0.26 μM of adenyl-methionine with a corresponding formation of 0.14, 0.22 and 0.25 μM of ATP and the incorporation of 0.13, 0.21 and 0.24 μM of $P^{32}P^{32}$ into ATP. These enzyme preparations also catalyze the formation of ATP from the adenyl derivatives of even those amino acids which do not themselves significantly promote the exchange of $P^{32}p^{32}$ with ATP (reaction 1).

659. INCORPORATION OF HYDROXYPROLINE INTO PROTEIN. C. R. A. Berger (intr. by L. M. Henderson). Radiocarbon Lab. and Div. of Animal Nutrition, Univ. of Illinois, Urbana.

Radioactive L-hydroxyproline (prepared enzymatically from the synthetic DL-compound by Drs. Meister and Radhakrishnan, to whom the authors are greatly indebted) was fed to 2 young (37 gm) rats, 1 of which was also given 20% nonradioactive proline, for 3 days. It was found that the free hydroxyproline in liver had been slightly diluted (6-fold) by an existing small nonradioactive hydroxyproline pool, more so on feeding proline (68-fold). An appreciable amount of the radioactive hydroxyproline was incorporated into protein. However, the specific radioactivity of the

hydroxyproline laid down in carcass protein during the 3 days was decreased nearly 2000-fold in the rat not receiving proline. This result supports the hypothesis of Stetten (J. Biol. Chem. 181:31, 1949) that hydroxyproline is not incorporated into protein from a free pool but formed in a bound state. No evidence for the presence of pyrrolecarboxylic acid (a metabolite of DL-hydroxyproline) could be detected in urine, showing that this compound is derived exclusively from D-hydroxyproline in vivo. Carnitine, a possible metabolite of hydroxyproline, was isolated and found to be nonradioactive.

660. METABOLISM OF CORTISOL 4-C ^{14} IN HUMANS. David. L. Berliner and Martha L. Berliner (intr. by Alejandro Zaffaroni). Dept. of Anatomy, Univ. of Utah College of Medicine, Salt Lake City.

The recent development of an isotope technique which allows both identification and quantitation of sub-microgram quantities of a large number of steroid metabolites (Fed. Proc. 15:219, 1956; Anal. Chem. 28:1608, 1956) has made it possible to study the metabolism of physiological quantities of cortisol in the human. One and two-tenths microcuries of 4-C^{14} cortisol were injected into a normal male subject. Several samples of blood and urine were obtained at various intervals and analyzed separately to determine metabolic patterns in respect to time. The following steroids were isolated from blood: cortisol; cortisone; 4-pregnene 11 beta, 17 alpha, 20 beta, 21 tetrol, 3-one; and 4-pregnene 11 beta, 17 alpha, 20 alpha, 21 tetrol, 3-one. Cortisone was found in the blood as early as 15 min after the injection. At 30 min both 4-pregnene 11 beta, 17 alpha, 20 beta, 21 tetrol, 3-one, and 4-pregnene 11 beta, 17 alpha, 20 alpha, 21 tetrol, 3-one were found and identified. Among numerous compounds isolated, 7 have been identified from urine at this time: cortisol; cortisone; 4-pregnene 17 alpha, 20 beta, 21 triol, 3, 11 dione; 4-pregnene 11 beta, 17 alpha, 20 beta, 21 tetrol, 3-one; 4-pregnene 11 beta, 17 alpha, 20 beta, 21 tetrol, 3-one; and compounds which lack hydroxyl group at the 21 position, were isolated from the free fraction. After a beta glucuronidase hydrolysis tetrahydro cortisol, tetrahydro cortisone, and three C-19 steroids were isolated. A progressive decrease in the ratio of amounts of high polar to low polar steroids was observed for most of the conjugated compounds. The lack of presence of C-19 steroids with intact ring A in the free fraction isolated from urine and blood, and the later appearance in urine of these steroids in the conjugated form, ascertains the fact that the loss of the side chain occurs after changes have taken place in ring A for conjugation. (Supported by Public Health Service Grant #C-2349(C), Cooperative Leukemia.)

661. PROPERTIES OF PURIFIED HUMAN SERUM β-LIPOGLOBULIN. Peter Bernfeld. Depts. of Surgery and Biochemistry, Tufts Univ. School of Medicine, Boston, Mass.

β-Lipoglobulin has previously been reported to be the only serum protein to precipitate with sulfated amylopectin. This property is being used for the quantitative determination of this protein, whereby the amount of precipitate formed at high dilutions is measured nephelometrically. Based on the same protein-polyanion interaction, β-lipoglubulin has been purified as follows: 1) precipitation of β-lipoglobulin by addition of sulfated amylopectin to serum; 2) removal of adhering serum proteins from the precipitate by dissolving it in 18% saline and reprecipitation of the complex upon dilution; 3) removal of the polyanion as insoluble barium salt, after dissolving the precipitate in 18% saline; 4) elimination of excess barium by ion exchange. When stabilized by 0.01 M pyrophosphate, β-lipoglobulin solutions thus obtained are stable; in the absence of pyrophosphate, they become rapidly turbid and lose their ability to precipitate with sulfated amylopectin. The purified lipoprotein has the same chemical composition as preparations obtained by other methods; it migrates as a single peak on electrophoresis at pH 8.6; it is precipitated by sulfated amylopectin and forms typical soluble complexes with heparin and sulfated polyuronates. At high dilutions, its ability to form insoluble complexes with sulfated amylopectin is markedly enhanced by a great variety of substances, notably by proteins, while no enhancement was observed with more concentrated solutions, or with diluted solutions of less pure lipoprotein, e.g. with serum.

662. BIOSYNTHESIS OF DEOXYRIBOSE. I. A. Bernstein and Dorothy Sweet.* Dept. of Dermatology, Med. School, Univ. of Michigan, Ann Arbor.

The tracer technique is being employed to investigate the over-all mechanism of the biosynthesis of deoxyribose (DR) in intact microorganisms and in animal tissues in vivo. Thymidine was isolated from the nucleic acid of Escherichia coli, strain B, grown on C^{14}-labeled glucose as the sole carbon source, and was degraded to determine the pattern of radioactivity in DR. The nucleoside was converted by fermentation with E. coli, ATCC 9723 (Hoffman and Lampen. J. Biol. Chem. 198:885, 1952), to thymine, $HOCH_2CH_3$ (C-1,2), CO_2 (C-3) and $HOOCCH_3$ (C-4,5) and each carbon of the alcohol and acid oxidized separately to CO_2. Preliminary data obtained by chemical degradation of labeled thymidine indicate that these fermentation products represent the carbons of DR in the order shown in parentheses. DR synthesized from glucose-3,4-C^{14} was labeled primarily in carbons 2 and 3. The ratio of specific activity per millimole of DR to that of glucose was 0.9. From glucose-2-C^{14}, the relative specific activities of carbons 1 through 5 were 100, 37, 10, 65 and 0. When glucose-1-C^{14} was used, no single carbon of DR had more than 2% of the activity originally present in carbon 1 of the labeled hexose. The results of these experiments suggest that a considerable portion of DR may arise directly from hexose by loss of carbon 1 although other pathways appear to be involved in the synthesis of this compound. A similar approach is being used with rat and mouse liver and ascites cells.

663. ENZYMATIC SYNTHESIS OF DEOXYRIBONUCLEIC ACID (DNA). Maurice J. Bessman,* I. R. Lehman,* E. S. Simms* and Arthur Kornberg. Dept. of Microbiology, Washington Univ. School of Medicine, St. Louis, Mo.

An enzyme from Escherichia coli which polymerizes deoxyribonucleotides (Biochim. et biophys. acta 21:197, 1956) has now been purified 1000-fold. The enzymatic reaction requires the triphosphates of thymidine (TPPP), deoxyadenosine (APPP), deoxyguanosine (GPPP) and deoxycytidine (CPPP), polymerized DNA and Mg^{++}. The reaction observed with P^{32}-labeled substrates is provisionally formulated as: $n(TP^{32}PP + GP^{32}PP + CP^{32}PP + AP^{32}PP) + DNA \rightleftharpoons DNA\text{-}(TP^{32}\text{-}GP^{32}\text{-}CP^{32}\text{-}AP^{32})_n + 4(n)PP$. Thymidine diphosphate cannot replace thymidine triphosphate. Omission of any one of the four triphosphates from the reaction results in rates of only 1% of that of the complete system; with a more sensitive

assay system, incorporation of single nucleotides into the product has been established and the kinetics are under study to provide a better description of such limited reactions. Polymerized DNA of animal, bacterial or viral origin serves in the synthetic reactions. In the absence of DNA, or upon its replacement by acid-treated DNA (apurinic acid) or RNA, no reaction is detectable (< 0.1%). The DNA product enzymatically synthesized from C^{14}-TPPP was shown to contain C-T and T-T dinucleotides in which C^{14}-thymidylate was present in typical 3'-5'-phosphodiester linkage. The sedimentation characteristics of the DNA product (kindly analyzed by Dr. H. K. Schachman) were not distinguishable from those of the added DNA. Inorganic pyrophosphate (PP) is released in amounts equimolar to deoxynucleotide incorporation. Reversal of the reaction by high concentrations of PP is indicated by an exchange of labeled PP with deoxynucleoside triphosphates in the presence of DNA, enzyme and Mg^{++}.

664. ESTIMATION OF CITRIC ACID AND KETONE BODIES BY THE SALICYLALDEHYDE-ACETONE REACTION. Samuel P. Bessman and Martin Anderson.* Univ. of Maryland Med. School, Baltimore, Md.

The highly specific salicylaldehyde-acetone reaction has been applied to the determination of the citric acid and total and differential ketone bodies. The citric acid is oxidized to acetone with vanacid acid and the acetone is microdiffused into a bisulfite solution. Alkali and salycylaldehyde are added and the color determined spectrometrically. The oxidation, diffusion and color development are performed in a standard screw-capped test tube. Recovery of citric acid is quantitative and the range of normal values is 13-27 gamma/ml of plasma. None of the substances which interfere with the pentabromoacetone method interfere with this determination. Total and differential ketones are determined directly on blood filtrates by oxidation with sodium dichromate and direct addition of salycylaldehyde-alkali reagents. No autoclave is required and both the ketone method and citric acid method are performed in screw-capped pyrex tubes requiring no transfers until colorimetry is performed. The ketone method is also quite specific and recovery of betahydroxy butyric acid and the acetoacetic acid are quantitative. As in other methods involving basic precipitation, acetone is recovered variably. The details of both methods will be presented and the specificity of the reactions demonstrated. (Aided by grants from the Natl. Science Fndn. and the Playtex Park Fndn.)

665. EFFECT OF HYPOPHYSECTOMY AND ADRENALECTOMY ON ESTRADIOL CONTROL OF DPNH OXIDATION IN RAT UTERUS. Arley T. Bever and Phillip J. Campbell (intr. by Alton C. Kurtz). Depts. of Biochemistry and Pharmacology, Univ. of Oklahoma School of Medicine, Oklahoma City.

Previous research (Endocrinology 58:512, 1956) demonstrated that estradiol-17B will elevate uterine DPNH oxidase activity in ovariectomized rats. It was observed then that an elevated enzyme level could not be maintained by constant daily injection of estradiol. A maximal activity in 3 days was followed by a minimal activity in 5-6 days which approximated castrate nontreated values. Experiments were made to study the effect of 1) hypophysectomy, 2) adrenalectomy and 3) combined hypophysectomy and adrenalectomy upon this enzymatic phenomenon in ovariectomized rats. None of the above treatments prevented or augmented the rise of the enzyme activity to the usual values of ovariectomized animals receiving the estrogen. None of the treatments prevented a subsequent fall in activity after a maximum value was reached. Adrenalectomy significantly increased the time required to obtain both the maximum and minimum activity values and this same effect was also observed when both hypophysectomy and adrenalectomy were performed prior to estrogen administration. It is concluded that the enzyme response to daily estradiol administration in ovariectomized rats is not primarily dependent upon the pituitary or adrenal glands, although the latter may have a modifying influence. These findings strengthen the probability that the decline phase of the response is due to the effects of metabolic products of estradiol metabolism. (This research was partly supported by an institutional grant from the American Cancer Society and principally supported by a grant from the Natl. Insts. of Health.)

666. ASSAY AND PURIFICATION OF MUTAROTASE FROM PENICILLIUM NOTATUM. D. S. Bhate* and Ronald Bentley. Dept. of Biochemistry and Nutrition, Grad. School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

A convenient chemical assay for mutarotase has been developed by using the specific oxidation of β-glucose by bromine. Mutarotase preparations are incubated with freshly prepared, 1.0% α-glucose in 0.1 M phosphate buffer, pH 5.6, for 20 min at 20°. The β-glucose produced is oxidized by immediate addition of an aliquot of the incubation mixture to an ice cold, 40% saturated solution of bromine in 0.4 M phosphate buffer, pH 5.6. After standing for 8 min at 0°, excess bromine is removed with corn oil and the residual glucose is determined. Using pure glucose anomers under these oxidation conditions, 80% of β-glucose is oxidized but only 4% of α-glucose. Suitable corrections can be made for this incomplete oxidation of β-glucose, and for spontaneous mutarotation (determined with a boiled sample of enzyme). Mutarotase is obtained by fractionation of culture fluids of P. notatum grown for 14 days on Czapek-Dox medium containing 2% corn steep liquor. The following steps give a 10-fold purification with 50% recovery of activity: 1) precipitation with aluminum sulfate and extraction of the precipitate with 0.2 M phosphate buffer, pH 5.6; 2) treatment at 20° and pH 9.2 for 30 min to inactivate notatin (glucose oxidase); 3) addition of ammonium sulfate to 34% saturation and removal of an inactive precipitate; 4) final precipitation with aluminum sulfate and extraction into 0.2 M phosphate buffer, pH 5.6. The final preparation migrates as a single peak on ultracentrifugal analysis and produces 100 μmoles of β-glucose per milligram protein per 20 min.

667. A NEW METHOD OF PREPARATIVE ELECTROPHORESIS. M. Bier. Dept. of Organic Chemistry and Enzymology, Fordham Univ., New York City.

While at present there are available several useful methods for preparative electrophoresis, they are all limited to rather small volume capacity. This limitation is overcome in the Free-Boundary Flow-Electrophoresis, which provides for the first time the means for large scale fractionation, concentration or purification of proteins and other colloids. The present laboratory model of the apparatus is compact and simple. It is made of Plexiglass and semipermeable membranes. The solution to be fractionated is exposed to a strong direct current electric field (1-10 v/cm) while passing through a system of 3

parallel semipermeable membranes in proper arrangement and the separation is effectuated in the free-flowing vertical column utilizing the laminar flow of the liquid. In one mode of operation, the protein components, or other colloids, are separated into two fractions, one containing the proteins at or near the isoelectric point, while the other fraction contains all the charged protein components. The method was applied to the single step preparation of human gamma globulins from plasma, flow rates of up to 4 ml/min having been realized. Parallel arrangement of a number of cells may increase this yield to practically significant quantities. The process can also be applied to the concentration of proteins or other charged colloids, whereby the extent of the concentration realizable is limited mainly by their solubility.

668. A CO-CARCINOGENIC FRACTION FROM SESAME OIL. Fritz Bischoff. Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.

In this laboratory, injection of certain cholesterol oxidation products dispersed in sesame oil produced local subcutaneous mouse fibrosarcomas; aqueous vehicles produced negative results. (Cancer Res. 6:403, 1946; Abst. Am. Chem. Soc. March, 3-C, 1954; Fed. Proc. 14:183, 1955; J. Am. Chem. Soc. 77:3928, 1955). In experiments (32-33 mice each) carried to the age of 18 months, 6 β-hydroperoxy-Δ^4-cholestene-3-one (I) produced a 66% incidence of fibrosarcoma (sesame oil vehicle); a 13% incidence (cotton seed oil vehicle). For α-cholesterol oxide (II) the fibrosarcoma incidence were 48% (sesame oil), 24% (cotton seed oil). The addition of sesamin (M.P. 121°C) to the cotton seed oil vehicle reduced the incidence of fibrosarcoma for (I) to 6%, for (II) to 9%. In experiments carried to the age of 15 months, the addition of the mother liquor of a sesame oil extract (U. S. patent 2467903-4) with (II) to cotton seed oil. produced a 33% incidence of malignant tumors. At this age fibrosarcoma incidence for (II) was 39% (sesame oil), 18% (cotton seed oil), 9% (cotton seed oil plus sesamin). These results suggest that the co-carcinogenic fraction of sesame oil is in the mother liquor of the sesamin concentrate, but is not sesamin which may actually be anticarcinogenic. The induced fibrosarcomas are highly malignant: mitotic figures, pleomorphism, invasion, rapid growth on transplantation. (Supported by Grant C1586, Natl. Cancer Inst., PHS.)

669. EFFECTS OF SODIUM FLUORIDE ON PHOTOSYNTHESIS. Norman I. Bishop (intr. by H. Gaffron). Research Insts. Univ. of Chicago, Chicago, Ill.

It is well known that enolase, catalase and perhaps other Mg containing enzymes are poisoned by fluoride. Fluoride inhibition of photosynthesis has been observed but the results were difficult to interpret because fluoride penetrates into unicellular algae only slowly under so-called normal physiological conditions. Warburg et al. have recently shown that the penetration is rapid for cells grown at acid pH (3.7-4.0). Under such conditions an inhibition of photosynthesis is often preceded by a sudden evolution of carbon dioxide. Certain carbohydrate transformations are coupled with the reactions leading to the assimilation of carbon dioxide. We have now investigated whether fluoride interferes with this particular type of carbohydrate metabolism. A few of the results are: The total amount of carbon dioxide released by fluoride from green cells under anaerobic conditions is related to the total fermentation activity of the cells and diminishes in proportion to the amount of fermentation. Long periods of fermentation cause the disappearance of the carbon dioxide burst. Thus the amount of carbon dioxide produced as a consequence of sodium fluoride can be varied at will. Using the chlorophyll content of the plant as a relative measure of the size of the burst as, for instance, Warburg et al. have done, we obtain from 0 to 3.5 times that expected. Preillumination of algal suspensions in white light under pure nitrogen increases the burst size. A period of photosynthesis prior to the addition of fluoride decreases the burst size. Alkaline or neutral grown cells resuspended for several hours in acid medium do not show the fluoride-induced carbon dioxide burst. Thus the observed effects of fluoride on photosynthesis appear to be concerned with a fermentable carbohydrate that may be involved in the assimilation of carbon dioxide.

670. A NEW NATURALLY OCCURRING THIOL AND OTHER SULFUR-CONTAINING COMPOUNDS ISOLATED FROM YEAST. Simon Black, Phyllis F. Downey* and Edith C. Wolff.* N.I.H., Bethesda, Md.

When yeast cells are incubated briefly with $CH_3S^{35}H$ a large number of compounds within the cells become labeled with S^{35}. The major known sulfur compounds, however, such as cysteine, glutathione and methionine, do not become labeled. The majority of the labeled substances appear to be previously unsuspected sulfur-containing amino acids or amino acid derivatives which are present in very small quantities. One of these substances has been identified as S-methyl cysteine and another as a new isomer of β-methyl lanthionine ($[\alpha]_D^{20}$ + 37.6° in HCl). When the yeast is allowed to grow most of the labeled compounds which can initially be extracted become "bound" and after 15 hr are no longer extractable with acid or heat. One substance, however, unlike the others, remains in a free state and can still be extracted with its S^{35} label after a period of growth. The latter substance has been isolated as a crystalline mercuric chloride complex having the empirical formula, $Hg_2Cl_2C_9H_{18}N_2SO_5$. One gram of the complex was obtained from 1 kg of fresh yeast. The mercury-free material gives a strong nitroprusside reaction for the -SH group. Chromatographically and analytically it is distinct from known thiols. Its concentration in the cell suggests that it may have a coenzyme function, a possibility which is being investigated together with studies on its chemical structure.

671. IN VITRO EFFECTS OF ADRENOCORTICAL STEROIDS ON TRANSAMINATION AND OXIDATION BY CELL SUSPENSIONS OF RAT THYMUS AND LYMPHOSARCOMA. Melvin Blecher* and Abraham White. Dept. of Biochemistry, Albert Einstein College of Medicine, New York City.

In vivo administration of certain 11-oxygenated adrenocortical steroids is known to cause marked histological and biochemical alterations in lymphoid tissue. Possible sites of action of these hormones were investigated by studying their in vitro effects on aspartic - α-ketoglutaric transaminase and on substrate oxidation in lymphocyte suspensions from male rat thymuses and Murphy-Sturm lymphosarcoma. Effects of hydrocortisone (HC) and cortisone were influenced by age of animal, gas phase during incubations, adrenalectomy and steroid concentration. Anaerobically, HC inhibited transaminase activity in thymic lymphocytes from prepubertal, and augmented activity in thymic lumphocytes from postpubertal, animals. Both effects were biphasic with hormone

concentration, were qualitatively similar in thymuses from adrenalectomized and normal rats and were not seen with desoxycorticosterone (DOC), testosterone or 17 α-ethyl, 19-nortestosterone. Transaminase activity aerobically was as great or greater than anaerobically; HC caused a slight and DOC a marked inhibition of aerobic transaminase activity in tymuses from both age groups. Oxidation, endogenous or of added substrate, by lymphocytes from both pre- and postpubertal rats was inversely related to concentration of HC or DOC. HC had no effect on anaerobic transaminase activity by tumor cell suspensions, although causing the usual effects on thymus cells from the lymphosarcoma-bearing animal. Although HC had no effect on anaerobic transamination in supernatant fluids from thymus homogenates, treatment of lymphocytes from postpubertal animals with HC prior to homogenization caused marked augmentation of activity. These results suggest a possible direct action of adrenocortical hormones on oxidative and transamination enzyme systems in lumphoid cells.

672. ENZYMATIC HYDROLYSIS OF URINARY STEROIDS. Eric Bloch (intr. by R. I. Dorfman). Worcester Fndn. for Exptl. Biol., Shrewsbury, Mass.

The enzymatic hydrolysis of urinary 17-ketosteroids and estrogen conjugates by an aqueous extract of the intestinal fluid of the snail Helix pomatia L. (Henry and Thevenet, Bull. Soc. Chim. Biol. 34:886, 1952) was studied. Hydrolysis conditions resulting in maximum 17-ketosteroid release (90 ± 20% recovery compared to 7-min, 15% HCl acid hydrolysis at 100°C) were incubation of the sample with extract concentrations containing 1.0-1.5 x 10^5 Fishman units β-glucuronidase and 2.0-2.5 x 10^5 p-nitrophenolsulfate units sulfatase activity per 100 ml urine (1.0-1.5% v/v) at pH 5.2, 39° ± 1°C for 24 hr. Hydrolysis was done with crude extracts; β-glucuronidase and sulfatase were not separated. Normal adult human urine did not require the addition of acetate buffer. Boiling urines for 10 min or precipitation of phosphates and sulfates with 0.2 M $BaCl_2$ at pH 11 prior to incubation did not appreciably increase 17-ketosteroid yields. Incubations carried out at different pH concentrations indicated two plateaus of maximum 17-ketosteroid release: pH 5.0-5.8 and 6.5-8.0. However, the percentage of etiocholanolone, dehydroepiandrosterone and artifacts in the total 17-ketosteroid extract increased with increasing pH, the percentage of androsterone decreased. Sodium androsterone-sulfate was not hydrolyzed enzymatically. In a majority of normal adult male urines studied, 85-95% of the conjugated 17-ketosteroids were hydrolyzed enzymatically, acid hydrolysis releasing an additional 5-15%. In a small number of instances, only 60-75% of the total conjugated 17-ketosteroids and less than 40% of conjugated androsterone were enzyme hydrolyzable. Considerable variation was found in the excretion of dehydroepiandrosterone, 11-oxygenated-androsterone and 11-oxygenated-etiocholanolone among individuals. Enzymatic and acid hydrolysis (60 min, 15% HCl by volume, 100°C) liberated similar amounts of estrone, estradiol and estriol.

673. EFFECT OF GLYCOCYAMINE ON NONCOLLAGENOUS PROTEIN LEVELS OF RAT MUSCLE. R. M. Bocek,* C. H. Beatty, R. D. Peterson, H. H. Dixon* and E. S. West. Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.

The noncollagenous protein (NCP) content of dilute alkali extracts of muscle has been determined by the biuret reaction. It has been found that the intraperitoneal administration of glycocyamine and betaine can raise the NCP level of rat muscle (control rats 15.33 gm% NCP; glycocyamine-betaine treated animals 16.35 gm% NCP, P = < .01). This increase in NCP has also been demonstrated in Warburg experiments using diaphragms from adult female rats (18-hr fasted weight 200-230 gm). Quarter diaphragms were incubated in Tris buffered Krebs-Ringer medium with glycocyamine (11 mM/l) and fumarate (5 mM/l). In 22 paired experiments, an average increase of 0.56 gm% NCP was found, P = < .005. When hemidiaphragms from growing female rats, 18-hr fasted weights 120-180 gm, were used, no change in NCP was found (average difference -0.19 gm%, n = 9, P = > .10). An average of 0.57 gm% NCP was recovered in the medium after the 1 1/2-hr experimental period. (Aided by grants from Internat. Minerals and Chemical Corp. and Public Health Service A-213 and A-1157.)

674. RELATION OF RNA METABOLISM TO PROTEIN SYNTHESIS. Ernest Borek, Ann Ryan* and T. D. Price.* Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.

The methionine deficient auxotroph, E. coli K_{12} W.6 accumulates RNA during methionine starvation. These RNA enriched organisms are uniquely slow in synthesizing protein after the restoration of methionine. (Borek, Rockenbach and Ryan, J. Bact. 71:318, 1956). Moreover, such organisms cannot support the replication of bacteriophage (T_5). In a study of the cause of such laggard synthesis the composition and metabolism of the accumulated RNA were investigated. The RNA which had been deposited during the period of unbalanced synthesis differed neither qualitatively nor quantitatively from the RNA extracted from the organisms in logarithmic growth. However, in metabolism, as measured by the incorporation of P^{32} into RNA nucleotides there was a profound difference between the organisms in logarithmic growth phase and the organisms with unbalanced accumulation of the nucleic acid. If a value of 100 is assigned to the P^{32} incorporation during 45 min by organisms in logarithmic growth phase, then the corresponding value for the first 45 min after the restoration of methionine to the RNA enriched organisms is 4; and the value during the second 45-min period, at the end of which new protein synthesis is just discernible, is 45. These findings would seem to indicate that, if, as has been postulated, there is a seminal relationship between RNA and protein synthesis then the governing factor is not the amount of RNA in the bacterial cell, but rather the cell's capacity to synthesize new, or alter old, RNA.

675. EXPERIENCES WITH DUAL CHANNEL ULTRAMICRO FLAME PHOTOMETER. Phyllis A. Bott. Dept. of Physiological Chemistry, Woman's Med. College of Pennsylvania, Philadelphia.

An instrument using the principles described by Ramsay, Brown and Falloon (J. Exper. Biol. 30:1, 1953) but with differences in detail was built for the author by the American Electronic Laboratories of Philadelphia. Some of the innovations, due principally to Mr. John Busser of this company, are as follows: The straight-path sample carrier has been replaced by a motor-driven arm which carries the platinum loop into the flame and out again in a circular motion. A metal cylinder covers the sample until it reaches the flame. Burning time is governed by an easily adjustable timing device. A Bausch and Lomb stigmatic special grating spectograph makes possible the use of photomultipliers behind the exit light slits

without the necessity for an image converter. Compressed air and propane pressures are kept at 4 ± 0.02 inches of water during readings by means of regulators. With a finer platinum loop (suggested by Ramsay) and a longer burning time, the effect of Na on K readings reported earlier (Fed. Proc. 15:223, 1956) is eliminated. In the lowest range of 2 to 20 μμM in the sample the standard deviation amounts to ± 0.2 to 0.5 μμM for K and ± 0.1 to 0.2 μμM for Na. With larger amounts the percentage error decreases. Samples (0.2 μl) of diluted serum, renal tubule fluid or urine are taken for simultaneous determination of Na and K. (Aided by grants from the Life Insurance Med. Research Fund.)

676. INCORPORATION OF THE IMIDAZOLE CARBON ATOM 2 OF L-HISTIDINE INTO PURINES IN BACTERIA. Helen R. Bowser* and Boris Magasanik. Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.

The enzymatic degradation of α-L-formamidinoglutaric acid, an intermediate in histidine degradation, by cell-free extracts of histidine-grown bacteria has been shown to proceed by two distinct pathways. Extracts of Aerobacter aerogenes and Salmonella typhimurium convert this compound to glutamic acid and formamide, while those of Pseudomonas fluorescens and Pseudomonas aeruginosa yield glutamic acid, formic acid and ammonia. Using L-histidine-2-C^{14} it was observed that only the organisms capable of degrading L-histidine via formic acid could incorporate the imidazole carbon 2 into their purines. Degradation of the purines of P. fluorescens grown on L-glutamic acid supplemented with 0.5 μM/ml of L-histidine-2-C^{14} revealed that incorporation occurred primarily into carbon 8 (RSA, 38) and not at all into carbon 2. Formate-C^{14} (0.5 μM/ml) showed a similar pattern of isotope distribution but was a far less efficient purine precursor (RSA, 2.0). Glycine-2-C^{14}, on the other hand, contributed equally to purine carbons 2 and 8. Experiments employing L-histidine-2-C^{14} (0.5 μM/ml) with an unlabeled formate pool (20.0 μM/ml) were performed to determine whether free formate was an obligatory intermediate in the incorporation of the imidazole carbon 2 of L-histidine into purines. Although incorporation from histidine was markedly depressed (RSA, 3.6), the purines were twice as radioactive as they would have been had they obtained all the isotope from the free formate pool. This observation suggests that in addition to incorporation via free formate another mechanism involving a more direct transfer of the formyl group from a histidine intermediate may be operating.

677. MECHANISM OF ATP UTILIZATION FOR GLUTAMINE SYNTHESIS. P. D. Boyer and H. J. Fromm.* Dept. of Physiological Chemistry, Univ. of Minnesota, Minneapolis.

The mode of participation of ATP in many coupled syntheses, including that of glutamine, is poorly understood. A new hypothesis is advanced that glutamate specifically bound to the enzyme adds ammonia, and that this addition product is phosphorylated by ATP, then subsequently decomposes to yield glutamine and inorganic phosphate. In harmony with this tentative hypothesis are observations on the incorporation of P_i^{32} into ATP under equilibrium conditions with high concentrations of purified enzyme from peas. This incorporation is much more rapid than that of glutamate-C^{14} into glutamine, even though the P_i^{32} incorporation is dependent upon addition of glutamine or of glutamate and ammonia. Previous experimental observations are in agreement with the hypothesis; these include direct transfer of O^{18} from the glutamate carboxyl to P_i formed in net glutamine synthesis, the equality of the rate of ADP^{32} and P_i^{32} incorporation into ATP under essentially equilibrium conditions (Varner and Webster. Plant Physiol. 30:393, 1955), and the apparent inactivity of γ-glutamyl phosphate (Levintow and Meister. Fed. Proc. 15:299, 1956). The mechanism suggested may possibly operate in a wide variety of ATP dependent syntheses. Other related kinetic studies did not show any discernible compulsory order of participation of reactants in the catalysis, e.g. the K_m for glutamate was 5.9 and 5.4 x 10^{-3} at 0.01 and 25 x 10^{-3} M ammonia, and the corresponding V_{max} were 2.9 and 11.8 x 10^{-5} M per minute. Independent addition of reactants to the enzyme may explain the low velocities observable at equilibrium in this and other enzymic syntheses.

678. SELECTIVE INTRACELLULAR BINDING OF RADIATION PROTECTIVE AGENTS BY MAMMALIAN TISSUES. Reagan H. Bradford,* Raymond Shapira* and David G. Doherty. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

S, 2-Mercaptoethylguanidine·HBr (MEG), prepared from S, 2-aminoethylisothiuronium·Br·HBr, is effective in providing survival to mice when given intraperitoneally prior to an otherwise lethal dose of X-radiation (Proc. Soc. Exper. Biol. & Med. 89:312, 1955). Examination of a series of mercaptoalkylguanidines yielded several compounds with a wide range of effectiveness and correlated chemical structure with protective activity (Radiation Res., in press). Determination of the distribution of several effective S^{35}-labeled compounds established that the spleen, bone marrow and liver were the most highly-labeled tissues. Intracellular distribution was therefore investigated to determine whether any selective absorption occurred. Nuclear, mitochondrial, microsomal and soluble cytoplasmic fractions of the liver were prepared by the procedure of Hogeboom and Schneider, and the activity, dry weight and nitrogen content of aliquots of these fractions determined. When MEG-S^{35} was injected and the liver removed 45 min later, a preferential distribution of the activity was observed. When a pair of optical isomers—injected at the dose where the D isomer was effective and the L isomer ineffective—was examined, it was found that the activity of the microsomal fraction obtained with the D isomer was twice that of the L isomer. Doubling the L isomer dose (an effective protective level) increased the activity of the microsomal fraction to that of the D isomer. Similar results were obtained with C^{14}-labeled compounds. The major activity of all particulate fractions was rightly bound and could not be removed by dialysis against cyanide.

679. ISOLATION OF AN INTERMEDIATE IN THE NONENZYMATIC CATALYSIS OF THE BREAKDOWN OF p-NITROPHENYL ACETATE BY BENZOYLHISTIDINE METHYL ESTER. Arthur S. Brecher* and A. K. Balls. Dept. of Biochemistry, Purdue Univ., Lafayette, Ind.

An intermediate compound was detected by paper chromatrographic techniques during the course of hydrolysis of p-nitrophenyl acetate by benzoylhistidine methyl ester in 0.067 M phosphate buffer at pH 6.2. The intermediate compound was observed to migrate to the same extent as synthetic acetylbenzoylhistidine methyl ester. In a large-scale hydrolysis, the incubation mixture was extracted with benzene, and acetylbenzoylhistidine methyl ester was isolated from the reactants and products by fractional

crystallization with ethanol. It was shown to be identical with synthetic acetylbenzoylhistidine methyl ester by elementary analysis, mixed melting points and by the hydroxamic acid test. The formation of this intermediate presents an analogy to that of acetylchymotrypsin in the enzyme-catalyzed reaction. A comparison of the initial rates of hydrolysis of p-nitrophenyl acetate by histidine, benzoylhistidine, and benzoylhistidine methyl ester indicated that the relative rates were 1.0, 3.4, and 4.4, respectively. It was observed that alcohols such as ethanol, n-butanol and tert-butanol accelerate the hydrolysis of p-nitrophenyl acetate to the same extent, suggesting that their varying effects on chymotryptic hydrolysis of p-nitrophenyl acetate may be due to steric factors, possibly related to the specificity of the enzyme.

680. ACTION OF CARBON TETRACHLORIDE ON LIVER MITOCHONDRIA. William F. Bridgers,* Donald Abele* and F. Edmund Hunter Jr. Pharmacology Dept., Washington Univ., Saint Louis, Mo.

Several investigators (Judah and Christie, Dianzani, Recknagel) have suggested that CCl_4 damages liver by producing structural or permeability changes in mitochondria. This study compares biochemical and morphological changes produced by exposing isolated mitochondria to CCl_4 with earlier findings with phosphate and metal ions. Preincubation for 20 min at 30° in 0.33 M sucrose which has been 1/3 saturated with CCl_4 at 0° results in swelling and loss of DPN (β-OH-butyrate oxidation). DPN restores oxidation but not phosphorylation. EDTA or anaerobiasis protects against the loss of DPN. Thus it appears that the release of DPN is not the result of direct action of CCl_4 but secondary to some oxidative change which occurs more readily after CCl_4 has modified mitochondrial structure. The biochemical changes are similar to but not identical with those seen after preincubation with phosphate. Electron microscope studies indicate that under certain conditions marked alteration of mitochondrial structure occurs without loss of DPN, while under other conditions less extensive alteration of structure may be accompanied by loss of DPN. (Supported by grants from the Natl. Cancer Inst., PHS, and the American Cancer Society.)

681. VALUE OF GELATIN AS A NUTRITIONAL SUPPLEMENT. Myron Brin, Bernard L. Oser and Mona Oser. Food Research Labs. Inc., Long Island City, N. Y.

The addition of gelatin a) to basal diets containing protein of which only 6.6% is derived from animal sources but which vary in total protein level, viz, 11, 15 and 20% of the diet, or b) to basal diets containing 11% protein of which increasing proportions, viz, 6.6, 25.5 and 60.7%, are derived from animal sources, resulted generally in markedly increased growth of weanling rats. Efficiency of food utilization (EFU) and nitrogen efficiency (NE) were enhanced under these conditions by supplementation with gelatin despite its inherently low biological value. Supplementation of these diets with 2% gelatin elicited significant improvement in NE, and raising the level of supplementation to 5% even further improved weight gains and EFU. Moreover, gelatin supplementation of a diet containing 11% protein of which 93.4% was derived from corn and wheat resulted in increased growth and EFU beyond that observed from solely increasing the level of vegetable protein. Bearing in mind the currently accepted biological value of 27 for gelatin as compared to 65-70 for whole corn and wheat, these results are of interest in that they indicate the dietary protein conditions under which gelatin can make a positive contribution to the diet.

682. IONIC REQUIREMENT FOR PHOSPHORYLATION, ATP-P^{32} EXCHANGE AND ATPase IN MITOCHONDRIAL FRAGMENTS. J. Ramsey Bronk* and W. Wayne Kielley. Natl. Heart Inst., Bethesda, Md.

A submitochondrial particle exhibiting phosphorylation coupled to succinate or DPNH oxidation has been isolated from sonic extracts of rat liver mitochondria suspended in a solution of KF and phosphate at pH 7.0 (Biochim. et biophys. acta, in press). Associated with the phosphorylating activity there is also catalysis of an exchange between the terminal phosphate of ATP and inorganic phosphate, a process observed by a number of investigators with intact mitochondria and fragmented preparations. Both the phosphorylation and exchange reactions as well as ATPase showed an essentially complete dependence on magnesium ions. Optimal concentrations for all three processes appeared to be the same. A number of other ions have been tested for their ability to substitute for magnesium in the above reactions. Divalent ions of manganese, cobalt and iron will activate the three reactions. Divalent ions of calcium, zinc and copper, monovalent rubidium and trivalent iron would not activate. The exchange of the terminal phosphate of ATP for inorganic phosphate, the sensitivity of the reaction to such agents as dinitrophenol and other parallelisms such as those observed here have suggested that the exchange reaction represents a fragment of the phosphorylation process. With respect to this possibility we have observed that the rate of exchange can be markedly increased by first reducing the components of the electron transport chain. These results will be discussed in relation to a possible mechanism.

683. EFFECTS OF PARATHYROID EXTRACT ON CALCIUM AND SULFUR METABOLISM. Felix Bronner (intr. by R. M. Archibald). Rockefeller Inst. for Med. Research, New York City.

Ca-45 or S-35-sulfate was injected once intraperitoneally into 31-day-old rats. Starting 24 hr later, 50 units of parathyroid extract (PTE) were given twice daily for a maximum of 500 U. The S-35 levels in the blood, urine, pelt and liver were higher in the treated rats than in the controls. The differences were small but consistent. Neither chemical nor autoradiographic analyses, however, revealed differences in the levels of S-35 in the skeleton. In the case of calcium, the treated rats excreted 6-16 times more Ca-45 in their urine than the controls, but only 4-8 times more Ca-40. In one experiment, in which the same amount of Ca-45 had been administered over a 3-day period before administration of PTE was begun, treatment increased Ca-45 excretion by the same factor by which Ca-40 excretion had been increased. These observations are interpreted as meaning that PTE influenced the mobilization of the most revently deposited calcium. Autoradiographic and chemical analyses also showed that there was less Ca-45 in the metaphyses of tibias from the treated than in tibias from the control animals. The PTE may have had an effect on bone deposition as well as resorption, for in the cartilage plates of the treated animals the mature chondrocytes were more numerous and those closest to the trabeculae were more engorged than in the controls.

684. INCORPORATION OF ACETATE INTO SKIN STEROLS IN VITRO. S. C. Brooks* and C. A.

Baumann. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Slices of skin were incubated with $CH_3C^{14}OOH$, the sterols converted to the p-phenylazobenzoates, and the esters separated into the Δ^5 and Δ^7 fractions plus an additional slow-moving radioactive upper zone. The incorporation of acetate into the sterols of mouse skin increased for about 3 hr, when the specific activity of the Δ^7 fraction was about 12 times that of the Δ^5 fraction, while that of the slow moving zone was intermediate. The specific activities of the sterols from rat skin were less than the corresponding fractions from the mouse, the Δ^7 fraction being much more active than the Δ^5 fraction. The activity of the free Δ^7 fraction, a relatively minor component, was about 4 times that of the more abundant esterified Δ^7-sterols. The activity of the cholesterol fraction decreased on recrystallization and at least half of the activity appeared to be due to a "high-counting companion" similar to that found by others in liver. The high activity of the Δ^7 fraction remained high on recrystallization of either azoate or free sterol. The application of methylcholanthrene to the mouse 12 hr prior to incubation markedly diminished the incorporation of acetate into the Δ^7 fraction while the activity of the Δ^5 fraction was increased somewhat.

685. EVIDENCE FOR EXCRETION OF FORMIMINOGLUTAMIC ACID (FIGLU) ACCOMPANYING USAGE OF PTEROYLGLUTAMIC ACID (PGA) ANTAGONISTS IN MAN. Harry P. Broquist and A. Leonard Luhby.* Research Div., American Cyanamid Co., Pearl River, N. Y. and Dept. of Pediatrics, New York Med. College, New York City.

The glutamic acid (GA) activity of normal urine is principally glutamine. The GA activity of a series of normal and pathological human urine specimens when determined microbiologically with Lactobacillus arabinosus was much lower when the urine was sterilized by autoclaving rather than by Seitz filtration. This was expected since autoclaving destroys glutamine. However urine from a series of children with acute leukemia being treated with 4-amino PGA or 4-amino-10-methyl PGA did not decrease in apparent GA activity and in some instances increased. Since there is evidence that PGA deficient rats excrete FIGLU, children receiving PGA antagonists might be expected to have an altered PGA metabolism and possibly excrete FIGLU which would be measured in the autoclaved assay since FIGLU is degraded on heating to GA. Chromatographic evidence supporting this view has appeared (Broquist. J. Am. Chem. Soc. 78:6205, 1956) and was further substantiated as follows: 1 gallon of urine from a child with acute leukemia in remission and receiving 4-amino-10-methyl PGA was chromatographed on Dowex 50, eluted with 1% sulfuric acid, sulfate was removed, and a highly purified mercury salt (MS) was obtained by Neuberg precipitation. The predominant component of MS when converted to the free acid possessed the microbiological and chemical properties of authentic FIGLU. This study implies that FA may function in formimino group transfer in man.

686. ENZYMATIC FORMATION OF ACETYL PANTETHEINE. Gene M. Brown (intr. by L. G. Wesson). Div. of Biochemistry, Massachusetts Inst. of Technology, Cambridge.

Others have shown that certain acids are metabolized as the S-acyl pantetheine derivatives in the same way as the corresponding S-acyl CoA derivatives. The present work was undertaken to ascertain whether or not CoA is necessary for the enzymatic formation of acyl pantetheine. Cell-free extracts of Lactobacillus helveticus 80, prepared by sonic oscillation, acetylated pantetheine in the presence of acetyl phosphate (or acetate and ATP), K^+ or NH_4^+, and Na_2S (to keep pantetheine reduced). CoA, but no other SH compound, was also acetylated similarly. Products were measured colorimetrically by the formation of acethydroxamate. Removal of the small amount of CoA present in the extracts by treatment with Dowex 1 caused no loss in ability to acetylate pantetheine. Additions of CoA to the Dowex-treated extracts did not increase the rate of formation of acetyl pantetheine. A comparison of acetyl phosphate and acetyl CoA as substrates showed that pantetheine was acetylated 3-4 times more effectively by acetyl phosphate when either crude or purified (10-fold) enzyme preparations were used. In this experiment acetyl pantetheine formed was separated from unreacted acetyl CoA and pantetheine by paper chromatography and determined by microbiological assay with L. helveticus 80. These results indicate that pantetheine can be acetylated enzymatically without the intermediate formation of acetyl CoA and suggest that pantetheine may act as a coenzyme in certain reactions.

687. ROLE OF N-TERMINAL LYSINE IN ANTIGENIC STRUCTURE OF RIBONUCLEASE. Ray K. Brown, Lawrence Levine* and Helen Van Vunakis.* Div. of Labs. and Research, New York State Dept. of Health, Albany.

Bovine pancreatic ribonuclease (RNase) was deaminated by treatment with $NaNO_2$ for 7 1/2 min at pH 4.0 and chromatographed on an IRC 50 column at pH 6.20. Two fractions were obtained that were distinct from native RNase. Their chemical, immunologic and enzymic properties were similar. Mole ratios of 8.6 amino groups, by Van Slyke determination, and 8.2 ϵ lysine residues, by the FDNB procedure, were found. The peptide containing the N-terminal lysine was not recovered chromatographically following trypsin digestion of the performic acid oxidized fractions. About 0.1 mole of DNP end group, as yet unidentified, was present. The deaminated products fixed fewer CH_{50} in complement-fixation experiments and precipitated less antibody than native RNase. The effect was greater with rabbit antiserum taken after the first series of immunizing injections than after a later series. Enzyme activity at various ionic strengths depends on the concentration of cyclic cytidine phosphate or ribonucleic acid used as substrate. Ionic strength affects deaminated RNase and native RNase differently. Guanidination of 9 or 10 of the ϵ amino groups yielded a chromatographically homogeneous product possessing about 10% of the enzymic activity. Guanidination reduces reactivity with antibody only slightly.

688. ISOLATION AND DETERMINATION OF URINARY HYDROXYKYNURENINE. R. R. Brown (intr. by E. C. Miller). Cancer Research Hosp., Univ. of Wisconsin Med. School, Madison.

In the routine use of quantitative analytical methods for tryptophan metabolites in urine (Brown and Price, J. Biol. Chem. 219:985, 1956) the kynurenine fractions from the ion-exchange columns frequently became yellow on addition of nitrite and paper chromatograms of these fractions showed the presence of 3-hydroxy-L-kynurenine. These observations were used to develop methods for the isolation and determination of this compound in urine. Samples of the kynurenine fraction were treated with sodium nitrite and

ammonium sulfamate and read at 367 mμ using a sample without nitrite as a blank. The increase in optical density was a measure of hydroxykynurenine. Recovery of added hydroxykynurenine from the columns averaged 96.7%. Other known components of this fraction did not interfere spectrophotometrically and hydroxykynurenine was the only spot on paper chromatograms which reacted with nitrite to give a yellow color. Normal humans excreted 1.6-5.4 mg of of hydroxykynurenine per 24 hr and 4.9-13.4 mg after an oral dose of 2.0 gm of L-tryptophan. Subjects receiving pyridoxine antagonists excreted relatively large amounts of hydroxykynurenine after 2.0 gm of L-tryptophan. From these urines about 70% of the estimated hydroxykynurenine was isolated in chemically and spectroscopically pure form. It was not possible to isolate hydroxykynurenine from normal basal urine although it was readily detected by paper chromatography of concentrates of large volumes of the kynurenine fraction.

689. PRODUCTION OF A HYALURONIDASE BY A SOIL BACTERIA. Robert Brunish and John Findley (intr. by Joseph Nyc). Dept. of Physiological Chemistry, Univ. of California Med. Ctr., Los Angeles.

As part of a program designed to obtain enzymes capable of liquefying the vitreous body of the eye an organism which depolymerizes hyaluronic acid was obtained from soil. This bacterium appears to be E. freundii. To select bacteria with hyaluronidase activity, initially a medium containing hyaluronic acid (HA) and glucose as the sources of carbon and nitrogen was employed. After 2 weeks' incubation on this medium a subculture was made to a medium containing HA as the sole source of both carbon and nitrogen. A pure colony was obtained from this medium by passage to a Bacto agar plate containing 0.05% HA. Hyaluronidase activity was determined turbidimetrically. During the initial stages of incubation little enzyme activity was found in the medium. Although a concentrated suspension of intact cells showed little activity, lysis of the cells through sonic disintegration liberated enzyme. The enzyme could be precipitated at pH 5.0 with saturated ammonium sulfate. Activity was lost, however, by ethanolic precipitation or by heating at 60°C for 10 min. Dialysis did not appear to effect the enzymic activity. A preliminary comparison of this enzyme to those obtained from streptococci and from testes will be made. (Supported in part by Public Health Service Grant B-844 and by the National Council to Combat Blindness, Inc.)

690. TURNOVER RATES OF TISSUE CONSTITUENTS. Donald L. Buchanan. Radioisotope Service, VA Hosp., West Haven, Conn.

To measure the carbon turnover of tissues with respect to the food as a whole, a uniformly C^{14}-labeled diet was fed to mice for extended periods. The diet contained 55% uniformly labeled sucrose, 40% yeast grown on the sucrose, 4% salt, and 1% of unlabeled organic supplements. The animals were killed at intervals and the specific activities of the tissues were measured by proportional gas counting. These radioactivities, divided by the specific radioactivity of the diet, represented unequivocal measurements of the fractions of the total carbon in each tissue which had been replaced by dietary carbon during the time of feeding. The plasma proteins were nearly all replaced by dietary constituents within 10 days while the liver and gastrointestinal tract were 95% replaced in 3 and 7 weeks, respectively. Brain and muscle were slowest to be reconstituted, both being only two-thirds replaced during 2 months of feeding. Spleen, kidney and lung had turnover rates intermediate between liver and muscle. The slow replacement of muscle protein was ascribed either to slow transport of dietary amino acids to muscle or to slow synthesis of protein. To elucidate this, several of the free amino acids from a kilogram of rat muscle were isolated and counted. Although the rats had been fed the labeled diet for 32 hr, the specific activities of the amino acids were far below that of the diet but much higher than that of the protein. It is concluded that the slow turnover of muscle protein is the resultant of slow transport and slow synthesis.

691. BIOCHEMICAL CHANGES IN THE LIVER ASSOCIATED WITH KWASHIORKOR. Helen B. Burch, Guillermo Arroyave,* Ruth Schwartz, Ana M. Padilla,* Moises Behar,* Fernando Viteri* and Nevin S. Scrimshaw. Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, Mo. and Inst. of Nutrition of Central America and Panama, Guatemala.

During treatment of children suffering from kwashiorkor, no change occurred in the levels of 7 liver enzymes (Waterlow and Patrick). Since certain flavin enzymes are particularly sensitive in rats to protein, amino acid, or riboflavin deficiency, these seemed most likely to change in kwashiorkor. Measurements in liver biopsy samples of 6 enzymes, and 5 other substances were made on 13 Guatemalan children. During treatment the children were given milk with a varied diet. Samples were obtained initially and after 3-4 weeks' treatment from 6 children. In these riboflavin, glycolic acid oxidase, DPNH-dehydrogenase, malic dehydrogenase and transaminase showed no change relative to protein. In contrast, xanthine oxidase activity increased from 2.7 mM per kilogram of protein per hour before treatment to 6.3 after treatment; D-amino acid oxidase activity from 165 to 441; and oxidized pyridine nucleotides from 3.47 to 4.37 mM/kg protein. Liver protein increased from 119 gm/kg wet weight initially to 177. If this represents a true regeneration of protein, the new protein must have been enriched in xanthine oxidase and D-amino acid oxidase. Lipides decreased but cholesterol showed no change. Values for liver biopsy samples after treatment are close to those obtained with autopsy samples from children dying from causes not related to kwashiorkor. (Supported in part by funds from Natl. Science Fndn., Williams-Waterman Fund and World Health Organization.)

692. METABOLISM OF HUMAN LEUKEMIC LYMPHOBLASTS AND ITS NORMALIZATION BY METHOTREXATE AND 6-MERCAPTOPURINE. Dean Burk, John Laszlo,* James Stengle* and Kent Wight.* Natl. Cancer Inst., Bethesda, Md.

Clycolysis (acid production from glucose) and respiration of lymphatic leukemia cells were measured under physiological conditions in serum containing added glucose and bicarbonate and saturated with 5% CO_2 in air or N_2 in Summerson manometers. The white cells consisted almost exclusively of lymphoblasts with some abnormal forms but essentially no granulocytes, and were obtained at intervals from the blood of 3 selected patients with acute, nonremissive leukemia by atraumatic standing and settling of the red cells using added fibrinogen. Metabolic quotients typical of malignant tumors, and remaining relatively constant for many hours during measurement, were observed: Q^{N2}_{A} (anaerobic glycolysis) = 20 to 30

cmm. acid produced/mg dry wt/hr); $Q^{O_2}{}_A$ (aerobic glycolysis) = 10 to 20; and Q_{O_2} (oxygen consumption) = -4 to -8. In addition to the marked aerobic glycolysis obtained (contraindicated by several of the earlier workers using less definitive techniques), the most notable finding was of remarkably low respiratory quotients (Q_{CO_2}/Q_{O_2}) that were often less negative than -0.5 and even positive (i.e. suggestive of net carbon dioxide fixation rather than production). A "normalization of malignant metabolism" was obtained by treatment of the lymphoblasts with the well-known antileukemic agents methotrexate (amethopterin) or 6-mercaptopurine (100 ppm). The aerobic glycolysis was invariably reduced (even to zero), the rate of oxygen consumption usually raised and the anomalous respiratory quotients shifted to more negative values approaching -1. These metabolically normalizing actions of the antifolic agents employed appear to be referable to cytoplasmic particulate, rather than nuclear, sites (cf. Hochstein. Proc. Am. A. Cancer Res., April 1957).

693. UREA SYNTHESIS DURING AZO-DYE CARCINOGENESIS AS STUDIED IN THE ISOLATED PERFUSED RAT LIVER. William T. Burke* and Leon L. Miller. Depts. of Radiation Biology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.

The isolated perfused livers of normal rats and of rats on a 3'-methyl-dimethyl amino azo benzene (3'-MeDAB) diet have been used in studies of urea formation. When a mixture of all of the essential and non-essential amino acids is added to the perfusing blood, there is a depression of urea nitrogen production in the 3'-MeDAB livers to about 50% that of the normal livers. When glutamic acid replaces the mixture on an equimolar nitrogen basis, both normal and 3'-MeDAB livers show greatly decreased urea synthesis. This may be attributed to a failure of glutamic acid to penetrate the liver cell. When glutamine is used, one-half of the added nitrogen is converted to urea; this is true in both the normal and the 3'-MeDAB livers. If L-arginine is added, urea is produced in normal livers in an amount which is equivalent to one-half the nitrogen of the added arginine, in accord with the Krebs-Henseleit urea cycle. The 3'-MeDAB livers, however, still show a marked depression of urea synthesis — 50% in the presence of high (3 mM) concentrations of arginine, up to 75% when 3/4 mM arginine are added. It may be concluded that a biochemical impairment of urea synthesis occurs in the 3'-MeDAB livers, affecting arginase and perhaps transaminase activity, and that a pathway from glutamine to urea exists which is different from the Krebs-Henseleit urea cycle.

694. L-ARABINOSE ISOMERASE AND L-RIBULOSE KINASE IN LACTOBACILLUS PENTOSUS. D. P. Burma, Y. Takagi and P. Z. Smyrniotis (intr. by L. A. Heppel). Natl. Insts. of Health, Bethesda, Md.

The first steps in the fermentation of L-arabinose by L. pentosus are: 1) isomerization to L-ribulose and 2) esterification of L-ribulose. The isomerization of L-arabinose was first described by Lampen (Abstr. Proc. Am. Chem. Soc. 1954, 44c) in extracts of L. pentosus. The enzyme has now been purified about 12-fold from extracts of L-arabinose-grown cells by fractionation with ammonium sulfate and acetone. The final preparation is stable at -10°. The equilibrium has been measured polarimetrically and with the cysteine-carbazole reaction. With either L-ribulose or L-arabinose as substrate the equilibrium mixture contains about 10% of L-ribulose and 90% of L-arabinose and is little affected by temperature. K_m is 4×10^{-2} for L-arabinose and 1.1×10^{-4} for L-ribulose and the enzyme is completely inactive with other pentoses. A second enzyme found in extracts of L-arabinose-grown cells catalyzes the esterification of L-ribulose by ATP; purified preparations of this enzyme are inactive with L-arabinose. The affinity for L-ribulose is high and the reaction proceeds readily to completion. The reaction product contains phosphate esters of both ribulose and xylulose. The xylulose ester is active with phosphoketolase which converts D-xylulose 5-phosphate to acetyl phosphate and triose phosphate (Horecker et al. Fed. Proc. 1957). This indicates that the L. pentosus extracts contain the enzymes which convert L-ribulose phosphate to D-xylulose 5-phosphate. This conversion has been observed by Simpson and Wood (J. Am. Chem. Soc. 78:5452, 1956) in extracts of Aerobacter aerogenes.

695. INCORPORATION OF GALACTOSE INTO GALACTOLIPIDS OF BRAIN TISSUE. Robert Main Burton,* Mary Ann Sodd* and Roscoe O. Brady. Lab. of Neurochemistry, Natl. Inst. of Neurological Diseases and Blindness, Bethesda, Md.

It has been observed that both D-galactose-1-C^{14} and D-glucose-U-C^{14} can be incorporated into the neutral galactolipid (cerebroside) fraction of rat brain tissue in vivo. The age of the animal was found to be important for studying the incorporation of hexose into cerebrosides. Rats from 10-20 days old exhibited the greatest labeling of cerebroside by radioactive sugars. Younger or older rats incorporated little galactose-1-C^{14} or glucose-U-C^{14} into the galactolipid fraction. Maximum labeling of the cerebrosides occurred in approximately 8 hr after a single injection of the C^{14}-carbohydrate. Within 24 hr following the administration of the labeled sugar, the specific activity of the cerebrosides had decreased to about 30% of the maximum value. Enzyme systems are present in preparations of rat brain tissue which are capable of catalyzing the incorporation of glucose or galactose into galactolipids in vitro. Microsomes isolated from rat brain tissue have been shown to utilize glucose or galactose when supplemented with 90% pure adenosine triphosphate (ATP) for the formation of radioactive cerebrosides, while crystalline ATP (free of nonadenine nucleotides) was found to be ineffective. When the crystalline ATP and galactose-1-C^{14} was supplemented with uridine triphosphate or uridine diphosphoglucose, the hexose-1-C^{14} was incorporated. Replacement of the free sugar, ATP and uridine nucleotides by uridine diphosphogalactose-1-C^{14} produced a relatively higher percentage of radioactivity incorporated into the cerebroside. Cerebrosides obtained from some of these experiments have been degraded and the sugar moiety has been shown to possess almost all of the radioactivity.

696. CITRATE ACCUMULATION IN SLICES OF NEOPLASTIC TISSUES. Harris Busch, Joseph R. Davis* and Esther W. Olle.* Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

A marked increase in citrate concentration occurs in kidney, spleen, brain, heart, lung, thymus, and pancreas of the rat following injection of sodium fluoroacetate. Small increases in citrate concentration are found in testis, blood, and muscle and no increase in citrate concentration is found in a variety of transplantable rat tumors or in the liver of the

male rat (Potter and Busch. Cancer Res. 10:353, 1950). In the present studies, citrate accumulation was determined after tissue slices were incubated in Krebs-Ringer phosphate or bicarbonate buffers with a variety of substrates, including glucose, glutamine, pyruvate, succinate, fumarate, malate, alpha-keto-glutarate and oxaloacetate, and varying tensions of oxygen. Fluoroacetate (0.006 M) increased the citrate accumulation in systems containing a mixture of all of these substrates (0.006 M) from 8.5, 1.8, 2.5, 2.4, 5.8, 2.3, and 5.1 μM/2 hr to 35.0, 8.0, 5.1, 4.8, 7.4, 3.2, and 6.1 μM/2 hr/gm of kidney, spleen, diaphragm, Walker 256 carcinosarcoma, liver, Jensen sarcoma, and brain respectively. Kidney slices produced maximal quantities of citrate in the presence of fumarate or malate or a mixture of pyruvate and succinate. Liver slices produced maximal citrate accumulation in the presence of a mixture of pyruvate, oxaloacetate and fumarate or malate. Tumor slices accumulated maximal concentrations of citrate in a medium containing a mixture of 0.006 M glucose, glutamate, pyruvate, fumarate, and oxaloacetate. In media containing a mixture of bicarbonate, glucose and pyruvate, liver and tumor slices produced approximately maximal citrate accumulation. Increasing the oxygen tension from 5% to 20% enhanced citrate accumulation by a factor of 4.2, 2.0, and 1.3 in kidney, liver, and tumor respectively, while an increase in oxygen tension from 5% to 100% increased citrate accumulation by a factor of 16, 3, and 3 in kidney, liver and tumor respectively. These data indicate that slices of tumor and nontumor tissues accumulate citrate under favorable conditions and suggest that one or more substrates essential for citrate accumulation is lacking in tumors and liver in vivo. (This research was supported by grants from the American Cancer Society and the Jane Coffin Childs Fund.)

697. SOME CHARACTERISTICS OF RAT KIDNEY 5-HYDROXYTRYPTOPHAN DECARBOXYLASE. James A. Buzard,* Priscilla D. Nytch* and M. F. Paul. Research Dept. Eaton Labs. Div. of the Norwich Pharmacal Co., Norwich, N. Y.

Although a pyridoxal phosphate requirement for guinea pig or hog kidney 5-hydroxytryptophan (5-HT) decarboxylase has been suggested (Clark et al. J. Biol. Chem. 210:139, 1954), conclusive demonstration of such a cofactor requirement has not been made. Data are presented which demonstrate a pyridoxal phosphate requirement for rat kidney 5-HT decarboxylase. Further results indicate that thiol groups are not essential for activity of this enzyme. Effect of various cations is discussed.

698. EFFECT OF OXIDATION-REDUCTION MEDIATORS ON METABOLISM OF DIFFERENTIALLY LABELED GLUCOSE. G. F. Cahill, Jr.,* J. Ashmore, A. B. Hastings and S. Zottu.* Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.

Metabolism of glucose-1-C^{14} and glucose-6-C^{14} by rat liver slices and isolated rat diaphragm muscle has been studied in the presence and absence of certain oxidation-reduction mediators. Rat liver slices incubated in Hastings' high potassium medium with differentially labeled glucose as added substrate exhibit a 2- to 3-fold increase in the rate of oxidation of C-1 of glucose in the presence of 10^{-4} M pyocyanin and methylene blue. The rate of oxidation of C-6 of glucose is not appreciably altered in the presence of these reversibly oxidizable dyes. It was observed that the incorporation of C^{14} from glucose into liver glycogen and into long chain fatty acids was appreciably lowered (to less than 30% of normal) in the presence of both pyocyanin and methylene blue. In the isolated diaphragm incubated in Ringer-HCO_3 medium oxidation-reduction mediators increase the rate of oxidation of both C-1 and C-6 of glucose. Glucose uptake by both liver and muscle was unaltered in the presence of methylene blue and pyocyanin. Other oxidiable dyes varying in E'_o (pH = 7.0) from +0.115 to -0.18 were found to be effective to different degrees in increasing the rate of oxidation of C-1 of glucose in rat liver slices. From these observations it is apparent that the presence of oxidation-reduction mediators play an important role in the relative contribution of alternate pathways to the overall pattern of metabolism.

699. EFFECTS OF CHOLINE AND VITAMIN E DEFICIENCIES ON ENZYMATIC PROCESSES IN MUSCLE AND LIVER. Mary P. Carpenter,* Paul B. McCay* and Ranwel Caputto. Oklahoma Med. Research Fndn., Oklahoma City.

Since the enzyme system which mediates the utilization of creatine-phosphate appears to be particularly sensitive to vitamin E deficiency, a group of rabbits were fed a Mason-Harris diet and 1) glycolysis, 2) phosphate transfer from creatine-phosphate to hexosemonophosphate, and 3) the phosphoglucomutase reaction were studied. The results show that glycolysis increases from about three weeks, but phosphate transfer from creatine-phosphate decreases. Phosphoglucomutase activity also diminishes. Animals on the above diet exhibited, in addition to muscular dystrophy, fatty, fibrotic livers, and so in a second series, a diet with controlled deficiencies in both vitamin E and choline was fed. Analysis of the data from this group of animals shows a significant decrease in phosphate transfer only in rabbits with both deficiencies. Meanwhile, there is no significant difference in glycolysis, confirming that a decrease in phosphate transfer occurs even when glycolysis is normal or increased. Further studies were done (Fed. Proc. 15:311, 1956) on the defect of oxidative phosphorylation in liver mitochondria; in contrast to muscle, the deficiency of tocopherol alone was sufficient, as previously reported, to lower P/O significantly (with tocopherol, 2.72; no tocopherol, 1.85; for the difference $P < 0.2$). The effect of a combined deficiency of vitamin E and choline on P/O is not additive. On the contrary, the ratio is higher than in vitamin E deficiency alone possibly due to impairment of respiration. Adequate choline and tocopherol do not prevent occurrence of fatty livers.

700. CHARACTERIZATION OF THYROTROPIN PREPARATIONS FROM CATION-EXCHANGE COLUMNS. Mary E. Carsten* and John G. Pierce. Dept. of Physiological Chemistry, Univ. of California Med. Ctr., Los Angeles.

This paper reports further characterization of preparations of thyrotropin (5 U.S.P. U/mg as measured by uptake of P^{32} in chicks) prepared by cation-exchange (Pierce and Nyc. J. Biol. Chem. 222:777, 1956). Sedimentation studies at pH 4.5-8.4 show a single peak with a S_{20} of 2.5-2.9; a S_{20} of 2.1 was obtained at pH 2.5. In experiments where trichloroacetic acid was used in attempts towards further fractionation, active fractions with variable but lower sedimentation constants were obtained. Preliminary experiments with free and paper electrophoresis indicated no gross inhomogeneity. The boundary or zone observed had a low mobility towards the anode in the pH range 4.5-9.2 and was somewhat diffuse. At pH 8.0-9.2 biological activity was shown to be in the material forming the boundary or zone. A clear fractionation was achieved by counter-current

distribution between butanol and p-toluene sulfonic acid although some inactivation occurred. The material active by the P^{32} assay strongly favored the aqueous phase and contained carbohydrate. Another fraction strongly favoring the organic phase was essentially carbohydrate-free. It is of interest that the latter fraction possessed a pepsin inhibiting activity which was recently demonstrated in pituitary extracts at this institution (Hilliard and West, unpublished work). The material favoring the aqueous phase was free of this activity. (Supported by a grant from the Public Health Service.)

701. DENATURATION OF DEOXYRIBONUCLEIC ACID. Liebe F. Cavalieri and Barbara H. Rosenberg.* Sloan-Kettering Inst., New York City.

Potentiometric titrations of DNA have been carried out at temperatures between 1° and 75°C, and the pH range over which denaturation occurs determined by conducting back-titrations from different pH's. Irreversibility and a 40% increase in E_{260}(P) occur in the same pH range, the narrowness of which (~.3 pH U) indicates cooperative collapse of the molecule. Although the curves for completely undenatured and completely denatured DNA are almost independent of temperature, the number of groups which must be titrated before denaturation occurs is strongly temperature-dependent, varying from over 90% at 1° to none at 75° (in 0.017 M NaCl). Cleavage by acid or alkali and thermal cleavage therefore appear to be additive when carried out simultaneously. Denaturation by either or both methods occurs when the proportion of intact H-bonds drops below that required to maintain the original molecular configuration. Furthermore, at 1° guanine must be involved in denaturation. A mechanism consistent with this is proposed, whereby acid and alkali each directly break one H-bond per nucleotide pair. Addition of urea before titration corresponds to elevation of the temperature, suggesting that thermal denaturation is a consequence of H-bond formation between bases and water (or urea). Viscosity measurements on DNA in 0.2 M salt show that the molecule collapses only when it is irreversibly denatured. When the pH is lowered to 2.6 and the viscosity measured at 1°C there is only a very small, but reversible, decrease in relative viscosity over that at pH 6.1; when the same procedure is followed at 22°C, the relative viscosity decreases irreversibly to 1.00.

702. ASCORBIC ACID METABOLISM. P. C. Chan,* L. M. Babineau,* R. R. Becker* and C. G. King. Dept. of Chemistry, Columbia Univ., New York City.

Previous studies of ascorbic acid metabolism have shown that the carbon chain of the vitamin is extensively converted to respiratory carbon dioxide in the whole animal. Further experiments in guinea pigs have been carried out with ascorbic acid-2,3,4,5,6-C^{14} (I), ascorbic acid-1-C^{14} (II), and ascorbic acid-3,4, 5,6-C^{14} (III). In 1-hr liver slice experiments, 25% of the radioactivity of II appeared as carbon dioxide, but only negligible quantities were obtained from I. When I was used as substrate, radioactive L-xylose was isolated from the reaction mixture by the carrier technique. Radioactive purity of the pentose was established by crystallization to constant specific activity and by paper chromatography. Radioactive L-xylose was obtained also from 24-hr urine samples, and from experiments with liver homogenates and supernatant fractions. The conversion of ascorbic acid into a 2-carbon compound in addition to oxalic acid was studied by the isolation of hippuric acid from 24-hr urine samples. When II was used, 95% of the activity was found in the carboxyl of glycine; from I, 40% of the label was in the carboxyl group, indicating that a large portion of the glycine was derived from carbons other than 1 and 2. III yielded uniformly labeled glycine. These and data on the distribution of radioactivity in glucose obtained from glycogen after injection of I and III are all consistent with a pathway of ascorbic acid metabolism involving the formation of pentose, followed by known metabolic pathways.

703. SUCCINATE-LINKED PYRIDINE NUCLEOTIDE REDUCTION IN MITOCHONDRIA. Britton Chance and Gunnar Hollunger. Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.

Succinate causes a rapid reduction of intramitochondrial pyridine nucleotide (P.N.) identified by its 340 mμ absorption band. Upon ADP addition, such P.N. is rapidly oxidized and becomes reduced again as the added ADP is exhausted. The oxidation-reduction kinetics are appropriately synchronized with the increases and decreases of respiration. In guinea-pig kidney mitochondria, glutamate, fumarate, and αketoglutarate reduce (in state 4) only 1/4 as much P.N. as does succinate; the latter reaction is direct and rapidly malonate-sensitive, and not dependent upon appreciable succinate turnover. Other states of the mitochondria containing less energy-rich intermediates are less favorable to succinate-linked P.N. reduction, which is delayed by prior ADP addition and prevented by uncoupling agents and antimycin-A, and only slightly speeded by ATP. The thermodynamic requirements for the overall reaction are fulfilled by intramitochondrial energy-rich intermediates to such an extent that the reduction is not inhibited by succinate-fumarate ratios of unity. Amytal (1.6 mM) blocks the oxidation of P.N., and slows its reduction 10-fold, while flavoprotein and cytochrome are more rapidly reduced and electrons are transported directly to the cytochrome chain. Thus in the absence of amytal, it is probable that cytochrome b and/or flavoprotein are driven in reverse by energy-rich intermediates to cause P.N. reduction. Such P.N. can provide energy for its reduction by participation in oxidative phosphorylation. Consequently, nearly constant P/O values and respiration rates of succinate metabolism occur in the presence and absence of amytal. These results give direct support to reversibility of phosphorylation and to compartmentalization of P.N. that may be of significance in ion transport.

704. EFFECT OF VITAMIN B_{12} ON LIVER DIPHOSPHOPYRIDINE NUCLEOTIDE. Carol C. Chang,* Jeng M. Hsu,* Robert L. Davis* and Bacon F. Chow. Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, and VA Hosp., Baltimore, Md.

Separate communications report that vitamin B_{12} deficiency results in a decrease of glutathione which causes the reduction of carbohydrate catabolism and gluconeogenesis, and in an increase in CoA. To study the inter-related functions of the coenzymes in carbohydrate metabolism pertaining to vitamin B_{12} deficiency, diphosphopyridine nucleotide (DPN and DPNH) in the livers of B_{12} treated and deficient female and male rats was measured. Five experiments were conducted involving the use of 5 litters of B_{12} deficient rats born by mothers fed a basal soybean diet (BSD). For each study, 1 or 2 pairs of littermates were employed. Half of them continued to receive BSD while the other half, BSD supplemented with B_{12} (100 μg/kg). At 8 weeks of age, the livers of rats were

assayed spectrophotometrically for DPN and DPNH with the aid of alcohol dehydrogenase. Their B_{12} contents were estimated microbiologically in order to compare their states of B_{12} deficiency. Our results indicate that in all 5 studies with 7 pairs of littermates (females), concentration of liver DPN was greatly increased and DPNH was markedly decreased. Mean values of the ratio of DPN to DPNH for all rats were 3.6 ± 0.24 in deficient animals and 1.8 ± 0.14 in the controls. Total liver DPN + DPNH (per gram rat) was significantly increased in vitamin B_{12} deficiency. Similar results were obtained with male rats. Studies of the effect of repletion with B_{12}, the supplementation of glutathione and alloxanization will be also reported.

705. CONVERSION OF MYO-INOSITOL TO GLUCURONIC ACID BY A RAT-KIDNEY ENZYME SYSTEM. Frixos Charalampous and Chryssoula Lyras.* Dept. of Biochemistry, Univ. of Pennsylvania, School of Medicine, Philadelphia.

In the course of our studies on the biosynthesis and metabolism of myo-inositol it was observed that rat tissue preparations were able to utilize added inositol. The uptake of inositol in these systems was accompanied by the formation of reducing compounds. We have now been able to obtain an enzyme system from rat-kidney which catalizes the formation of a uronic acid from myo-inositol. The uronic acid was isolated by ion exchange chromatography and was shown to possess the following properties: it has reducing properties (Nelson Method) and with orcinol or naphthoresorcinol it produces colored products whose spectra are identical with those obtained with authentic glucuronic acid. On paper chromatograms, using 6 different solvent systems, it migrates as a single spot indistinguishable from authentic glucuronic acid. Aniline oxalate can be used to detect the spot on paper chromatograms, indicating the presence of an aldehyde group. When isolated under conditions favoring lactone formation the uronic acid is partly converted to glucuronolactone. The isolated uronic acid is free of phosphorus and has no ultraviolet spectrum. Other physical and chemical properties of the isolated acid and its derivatives will be presented. The kidney enzyme system has been purified with ammonium sulfate fractionation. It catalizes the above reaction in the presence of oxygen and loses easily its activity on storage at 5°. Data regarding cofactor requirements as well as the ability of the system to couple with artificial electron acceptors will be presented.

706. EFFECTS OF "TOTAL" PANCREATECTOMY ON THE BLOOD LIPIDS AND KETONE BODIES OF THE RAT. Sidney S. Chernick and Robert O. Scow. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

Removal of 99.5% of the pancreas of the rat produces severe diabetes (Scow. Fed. Proc. 1957). Death occurs within 48 hr unless insulin is administered. At autopsy the liver and kidney are invariably engorged with fat. The sequence of events following pancreatectomy is under investigation. Total blood lipids were determined by a modification of the method of Bragdon (J. Biol. Chem. 190:513, 1951) on 0.1 ml of blood. Ketone bodies were measured by a method suggested by Bessman on 0.1 ml of blood. Rats were fasted 17 hr before the operation. Sham-operated animals served as controls. Blood samples were obtained 2, 4, 8, 12, and 24 hr after the operation during which time no food was given. In some rats elevated blood glucose, lipid, and ketone bodies were observed by 2 hr; at 8 hr these values were high in all pancreatectomized rats. Twenty-four hours after removal of the pancreas blood glucose values were >200 mg% (controls, <80 mg%); total lipid values, >1000 mg% (controls, <500 mg%); and total ketone bodies, >80 mg% (controls, <20 mg%). Effects of various hormones on the level of blood lipids and ketones in the pancreatectomized rat will be presented.

707. COLORIMETRIC MICRO DETERMINATION OF SERUM CALCIUM. Max E. Chilcote and Richard D. Wasson (intr. by Wilson D. Langley). Dept. of Biochemistry, Univ. of Buffalo School of Medicine, Buffalo, N. Y.

The method suggested here is based on the observation of Gorsuch and Posner that the colors produced by addition of micro amounts of calcium to solutions of ammonium purpurate at pH 11 obey the Beer-Lambert law. Our procedure allows the determination of the calcium in 50 µl aliquots, or less, of serum simply by observing the change in optical density on rinsing the serum into cuvets containing cooled ammonium purpurate at pH 12. Altering the pH to 12 eliminates interference by serum magnesium, and cooling of the buffered dye solution increases the stability of the dye sufficiently to allow accurate analysis. A total elapsed time of 24 minutes is allowable for dissolving an ammonium purpurate-NaCl powder in dilute KOH at room temperature, pipeting of aliquots of this solution into cuvets chilled in an ice bath, and completion of colorimetric analysis. At least 5 serum aliquots may be analyzed within the 24 min. Statistical comparison of the results obtained from analyses of a number of serums by this and the Clark-Collip methods indicate excellent agreement between the two methods. Appreciably better precision in this series is obtained by the suggested method. The presence of abnormal amounts of bilirubin in serum does not interfere, but visible hemolysis leads to incorrectly high calcium values.

708. PURIFICATION AND PROPERTIES OF A GLUTATHIONASE. Gerald M. Christensen (intr. by F. Binkley). Div. of Basic Health Sciences, Emory Univ., Ga.

One of the major difficulties in the study of the hydrolysis of glutathione by kidney tissue preparations has been the lack of suitable methods of obtaining a soluble preparation and carrying out further purification. Considerable purification of the activity of porcine kidney responsible for the hydrolysis and the transfer of the gamma-glutamyl moiety from glutathione to suitable acceptor peptides and amino acids has been obtained by a procedure involving the addition of sodium lauryl sulfate and $MgCl_2$ to a kidney homogenate followed by incubation at 37° for 1 hr and at 4° for 16-24 hr. After centrifugation and filtration through Hy-flo, the resultant filtrate contains approximately 50% of the activity of the homogenate and can be further purified by acid precipitation and fractionation with ethanol or ammonium sulfate. By treatment with acidic buffers and dialysis, preparations of the glutathionase may be freed of detectable amounts of the enzymatic activity responsible for the hydrolysis of cysteinylglycine. Under these conditions, the addition of a highly purified cysteinylglycinase preparation is required for maximal activity. A study has been made of the properties of this enzyme with respect to requirements for optimal activity, behavior on strip and continuous flow paper electrophoresis, and products of the reaction under various conditions. On continuous flow paper electrophoresis 2 components have been obtained, neither of which is active

not exhibit full activity when recombined. Attempts to identify these 2 components will be reported.

709. CHEMISTRY OF CHONDROITINSULFURIC ACID B (β-HEPARIN). J. Anthony Cifonelli,* Julio Ludowieg* and Albert Dorfman. Depts. of Pediatrics and Biochemistry, Univ. of Chicago, and LaRabida Jackson Park Sanitarium, Chicago, Ill.

A mucopolysaccharide prepared from beef lung by Dr. A. Winterstein (Marbet and Winterstein. Helv. Chim. Acta 34:2311, 1951) appears to be identical with the chondroitinsulfuric acid B found in skin. Hydrolysis in 1.0 N HCl for 30 min at 100° resulted in a mixture of compounds as demonstrated by chromatography on paper in a neutral amyl alcohol solvent. The most rapidly migrating substance was shown to be a uronic acid. When this spot was rechromatographed in 2 different solvents, its mobility was identical with a synthetic sample of L-iduronic acid and L-iduronolactone. Reduction of this material with sodium borohydride gave a mixture of 2 compounds which were similar in migration in 2 different solvents with those obtained by the reduction of known L-iduronic acid. From the original hydrolysate 2 other spots were isolated each of which contained bound galactosamine. Both of these showed a 510 mμ absorption maximum in the Elson-Morgan reaction, before hydrolysis, but a 540 mμ maximum after hydrolysis. One spot was shown to contain uronic acid while the other contained no uronic acid. The absorption maximum at 510 mμ in the Elson-Morgan reaction has been shown to be correlated with 3-substituted hexosamine structure in this laboratory. (Work supported by a grant from the Natl. Heart Inst., PHS.)

710. INDUCED SYNTHESIS OF TRYPTOPHAN PEROXIDASE IN RAT LIVER SLICES. Morton Civen* and W. Eugene Knox. Harvard Med. School and New England Deaconess Hosp., Boston, Mass.

Tryptophan peroxidase-oxidase (TPO) in liver can be adaptively increased in a few hours by treatment of intact rats with tryptophan or cortisone (Knox and Auerbach. J. Biol. Chem. 214:307, 1955) or by perfusion of surviving rabbit liver with tryptophan (Price and Dietrich. Fed. Proc. 15:330, 1956). Activity measurements, C^{14}-valine incorporation into the TPO-containing fraction (Gros et al. Bull. soc. chim. biol. 36:1569, 1954), and ethionine inhibition of the adaptive response (Price and Dietrich. Ibid. Lee and Williams. Biochem. et biophys. acta 9:698, 1952) indicate that the increased activity is referable to increased synthesis of the enzyme protein. A similar net synthesis of TPO can be demonstrated in rat liver slices incubated in vitro for 2-4 hr at 37°, under certain conditions. In a medium similar to that used for tissue culture with added rat serum the initial TPO activity in fresh liver slices is maintained during aerobic incubation. With added L-tryptophan over 2-fold increases in the activity can be obtained. This simpler system has been used to investigate the ways in which the 2 sorts of effective stimuli, the substrate and certain hormones like hydrocortisone, induce the synthesis of this specific protein in intact animals. (Supported by the Natl. Insts. of Health, PHS, and the Atomic Energy Comm.)

711. ADRENAL STEROIDS AND CALCIUM METABOLISM IN THE RAT. Irwin Clark and Raymond F. Geoffroy.* Merck Inst. for Therapeutic Research, Rahway, N. J.

A marked increase in urinary excretion of radiocalcium was observed after the injection of a single s.c. dose of 11-oxygenated steroids 48 hr after the administration of radiocalcium. This increase began at about the 3rd hr and was maintained until the 8th hr, after which time the values fell to the level of the control animals. Daily injections of these steroids 50 days after the injection of radioactive calcium, again resulted in an increased urinary excretion of radiocalcium. In these experiments, the increased excretion of radiocalcium was not evident until the 3rd or 4th day and this increase was augmented as long as the steroid was administered. The effectiveness of the steroids in increasing radiocalcium excretion paralled their clinical and biological behavior in most instances; i.e., prednisolone > prednisone > hydrocortisone > cortisone. Pretreatment of adult male rats with hydrocortisone for 2-3 mo resulted in an increased urinary excretion and in a decreased bone uptake of radiocalcium as compared to control rats also fed ad libitum. A decreased uptake of radiocalcium by bone was also observed in similar groups of rats which were nephrectomized immediately prior to the injection of radiocalcium.

712. SYNTHESIS AND BIOLOGICAL PROPERTIES OF ANALOGS OF VITAMIN B. John F. Codington and Heinz M. Wuest (intr. by Irving Goodman). Sloan-Kettering Inst. for Cancer Research, New York City.

"O-Heterothiamine," (I), and "β-mercaptoethylthiamine," (II), were prepared as part of a program for the synthesis of potentially interesting analogs of thiamine. 4-Methyl-5-(β-hydroxyethyl)oxazole, (III), was prepared from 1-acetyl-3-bromopentanone-4 and formamide. III was condensed with 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide, (IV), and, after treatment with AgCl, gave the O-hetero analog, (I), of thiamine. Condensation of 4-methyl-5-(β-mercaptoethyl) thiazole with IV gave a mixture, which was separated into two major components using cellulose powder chromatography. Crystalline "β-mercaptoethylthiamine," (II), was obtained in 43% yield. II gave a weaker thiochrome reaction (fluorescence) than did thiamine; whereas I did not give a thiochrome reaction. The ultraviolet and infrared spectra of I were compared with those of thiamine. I is neither a substitute for, nor an inhibitor of, vitamin B_1 in Phycomyces blackesleeanus. In the presence of the thiazole moiety of thiamine, I was found to exhibit 10% of the activity of thiamine. Under the same conditions II gave 50% vitamin activity. (Robbins, Botanical Garden, Bronx Park, N. Y.) II inhibited the growth of L. fermentum in the presence of thiamine and exhibited 18% of thiamine activity in yeast fermentation. (J. Scheiner, Hoffman La Roche, Inc., Nutley, N. J.)

713. GLUCOSE INHIBITION OF RESPIRATION IN THE DEVELOPING RETINA. L. H. Cohen (intr. by C. A. Nichol). Roswell Park Memorial Inst., Buffalo, N. Y.

Respiration of tumor tissues is known to be inhibited by glucose (Crabtree effect). This is believed to result from a decrease in level of ADP and/or inorganic phosphate caused by a stimulation of glycolysis (Gatt, Krimsky and Racker. Fed. Proc. 15:259, 1956; Brin and McKee, Cancer Res. 16:364, 1956). If this explanation is correct, nontumor tissues should also exhibit a Crabtree effect if their aerobic glycolysis is high. No Crabtree effect is found with the adult rabbit's retina despite an aerobic glycolysis exceeding that of tumor cells. Study of the postnatal development of the retina, however, revealed that prior to the formation of the sensory elements of the visual cells glucose inhibits respiration as much as 40%.

Inhibition is abolished by 2,4-dinitrophenol. The disappearance of the Crabtree effect during development coincides with a) 4-6 fold increase in Q_{O_2} b) insignificant change in aerobic glycolysis and c) increased oxidation of added U-C^{14}-glucose from 30% of the oxygen consumption in the young retina to 90% in the adult retina. This and kinetic data indicate that the adult retina contains a highly active component of respiration which does not share its phosphorylative cofactors with a system of glycolysis of high capacity. This respiratory component appears to reside in the sensory elements ("inner limbs") and may be a special equipment for the primary processes of vision. (Supported by Public Health Service Grant B-812.)

714. MINOR CONSTITUENTS OF RIBONUCLEIC ACIDS. Waldo E. Cohn. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

Ion-exchange chromatograms of hydrolyzates of ribonucleic acids from various sources have, from time to time, shown the presence in the mononucleotide region of ultraviolet-absorbing, phosphorus-containing substances. The propensity of nucleic acid preparations to include and tenaciously retain a variety of impurities makes it necessary to prove in each case that these new substances contain the correct components in the usual array and that they are incorporated into the usual phosphodiester linkage in the macromolecule. For at least one such constituent, apparently a modified uridylic acid, most of these criteria have been met. It appears in a wide variety of yeast nucleic acid preparations as the conventional a and b forms after alkaline hydrolysis, as the b form after ribonuclease hydrolysis, and as a riboside after snake venom hydrolysis. Its position in chromatograms near uridylic acid and uridine, its pronounced bathochromic shift at pH ca. 10, and the absence of spectral shifts below pH 8 indicate that it is a uracil derivative. Its ultraviolet spectra at neutral and alkaline pH's are almost identical with those of uracil and thymine in shape, with maxima and minima lying between or near them. Inasmuch as the substance is a nucleotide and not a base, the similarity of its spectra to those of the free pyrimidine bases is most unusual and indicates that the pyrimidine ring is able to assume a form which uracil and thymine cannot after they are converted to their ribosides, or deoxyribosides, or otherwise substituted on the 3 nitrogen atom.

715. COMPARISON OF BACTERIAL AND MAMMALIAN HEXOSAMINE-PHOSPHATE DEAMINASES. Donald G. Comb* and Saul Roseman. Rackham Arthritis Research Unit and Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.

Previous reports have demonstrated that glucosamine-6-phosphate (Gm-6-P) was deaminated to yield fructose-6-phosphate and ammonia by extracts either of hog kidney (Leloir and Cardini, Biochim. et biophys. acta 20:33, 1956) or of Escherichia coli (Comb and Roseman, Biochim. et biophys. acta 21:193, 1956). The partially purified deaminase from kidney required N-acetylglucosamine-6-phosphate (N-Ac-Gm-6-P) for activity and showed a specific activity (S.A.) of 5 μM product/mg protein/15 min incubation. The deaminase from E. coli did not require N-Ac-Gm-6-P and exhibited S.A. of 1200. In the present studies: 1) kidney extract was purified over 1000-fold and the enzyme (HK) showed S.A. of 770, 2) E. coli extract was purified 120-fold and the enzyme (EC) shoed S.A. of 4,200. Assays were conducted chemically or enzymatically (isomerase, glucose-6-phosphate dehydrogenase, TPN). Both enzymes were noted to be greatly affected by the type and concentration of the buffer used. The enzymes were studied with combinations of analytically pure N-Ac-Gm-6-P, crystalline Gm-6-P, crystalline galactosamine-phosphate (Galm-P; presumably Galm-6-P), and N-acetylgalactosamine-P (N-Ac-Galm-P; presumably N-Ac-Galm-6-P). Studies with EC demonstrated that: 1) Gm-6-P deamination was slightly and nonspecifically stimulated by N-Ac-Gm-6-P, 2) Gm-6-P deamination was slightly inhibited in the presence of either Galm-P or N-Ac-Galm-P 3) Galm-P deamination was not detectable. Preliminary studies with HK indicated that: 1) Gm-6-P alone was not deaminated 2) mixtures of Gm-6-P and N-Ac-Gm-6-P (or N-Ac-Galm-P) were rapidly deaminated 3) N-Ac-Gm-6-P alone was slowly deaminated 4) mixtures of Galm-P and N-Ac-Gm-P (or N-Ac-Galm-P) were slowly converted to ketose-P.

716. BIOLOGICAL ACTIVITIES OF TOBACCO MOSAIC VIRUS NUCLEIC ACID. Barry Commoner, Eddie Basler, Jr.,* Mas Yamada* and Tung-Yue Wang.* Henry Shaw School of Botany, Washington Univ., St. Louis, Mo.

Tobacco mosaic virus nucleic acid is capable of initiating an infectious process after being isolated from the virus nucleoprotein. This activity is lost rapidly soon after isolation. This effect is associated with configurational changes in the RNA, and may be influenced by properties of the solution in which the RNA is maintained. The physical configuration of the RNA is also influenced by association with the virus protein, the latter apparently having the capacity to restore configurational changes which occur after RNA isolation. The relation between these phenomena and the biosynthesis of TMV, and its relation to host DNA will be discussed.

717. HEPATIC GLYCOGEN, LIPOGENESIS, AND GLUCOSE-6-PHOSPHATASE IN X-IRRADIATED RATS. John G. Coniglio, John C. Kirschman and Granville W. Hudson (intr. by William J. Darby). Biochemistry and Radiology Depts., Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Relationship between hepatic glycogen concentration and lipogenesis in X-irradiated fasted rats was investigated. Rats given 750r total-body X-irradiation and nonirradiated controls were fasted 1/2-18 hr, injected intraperitoneally with C^{14}-acetate, and killed 30 min later. Greater C^{14}-incorporation into hepatic fatty acids occurred in the irradiated group as early as 6 hr after irradiation. At this time the liver glycogen concentration in the irradiated group was lower than in the control. Before 18 hr consistently higher concentrations of hepatic glycogen were not observed in irradiated rats compared to controls. A second group of rats was given 750r total-body X-irradiation and fasted 24 hr. Ten minutes prior to irradiation half of these had been given a protective dose of S, β-aminoethylisothiouronium bromide. Blood glucose and hepatic glycogen concentrations did not increase in protected in contrast to nonprotected animals. However, the effect of the protective agent on hepatic lipogenesis was small or altogether absent. Apparently, liver glycogen content per se is not the sole factor contributing to increased hepatic lipogenesis in irradiated fasted animals. Since liver glucose-6-phosphatase reduces the amount of glucose-6-phosphate available for glycolysis, the effect of total-body X-irradiation on this enzyme was studied. An increase in activity of enzyme in irradiated rats was comparable to that found in nonirradiated rats fasted for a similar length of time. (Supported by Atomic Energy Comm. Contract No. AT(40-1)-1033).)

718. OXIDATION OF PHENYLPYRUVIC ACID. Eric E. Conn and S. Louise Seki.* Dept. of Agricultural Biochemistry, Univ. of California, Berkeley.

Mitochondrial preparations from higher plants catalyze a rapid oxidation of phenylpyruvic acid. Some details of this reaction have been determined using mitochondria from the cotyledons of etiolated lupine seedlings. The reaction is heat labile, and is inhibited by cyanide, catalase and low concentrations of catechol and hydroquinone. These results suggest that hydrogen peroxide may be an intermediate in the reaction. However, all attempts to demonstrate hydrogen peroxide in the reaction mixture during the course of the reaction have been unsuccessful. A study of the stoichiometry showed that one mole of oxygen is consumed for each mole of substrate oxidized with the formation of somewhat less than one mole each of benzaldehyde and carbon dioxide. There is no evidence for formic acid in the reaction mixture and added formate is not metabolized during the course of the reaction. The ortho, meta and para hydroxyphenylpyruvates are also oxidized, but at slower rates. The following keto-acids were inactive: pyruvic, glyoxylic, and phenylglyoxylic. In an attempt to determine the nature of intermediates possibly formed during the oxidation of phenylpyruvate, oxygen consumption and/or carbon dioxide production were followed when different compounds were incubated with lupine mitochondria. Under these conditions, phenylacetaldehyde was only slowly oxidized; mandelic, phenylglyoxylic, phenylacetic, oxalic and formic acids were inactive. Mitochondria from pea, mungbean, oat and soya bean seedlings catalyze the oxidation of phenylpyruvate. In addition, a mixture of peroxidase and manganous chloride is an effective catalyst for the reaction.

719. HEMINS OF BEEF HEART MUSCLE. Jerald L. Connelly,* Martin Morrison* and Elmer Stotz. Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

An attempt has been made to investigate the prosthetic groups of the insoluble heart muscle hemoproteins. Ground heart muscle is first washed nearly free of hemoglobin and myoglobin. Most of the lipid is removed by organic solvent extractions. The hemins are then cleaved from their protein moieties and are extracted by treatment with acidified acetone. A preliminary purification of the hemins is achieved by precipitation of the hemins from the acetone solution. The hemin precipitate is washed with acetone and water and then is dissolved in methanol. Following this the methanol is evaporated in vacuo and the hemins are dissolved in chloroform. Finally, the crude sample is chromatographed on a column of hydrated silicic acid. The eluent is collected automatically and the absorption at 410 mμ is plotted against the tube number to give a graph which indicates the number and relative quantities of the hemin components. In order of elution the constituents are: (I) a minor red fraction; (II) a major red fraction; (III) a green fraction; (IV) a second minor red fraction; (V) a large red fraction; (VI) a brown fraction; and (VII) a large brown fraction. These compounds have been identified both by spectral absorption and by paper chromatography using 4 different solvents. Fraction II has been reported as a lipid containing hemin. Fraction III is believed to contain degradation products of the hemins a. Fraction V is protoheme. Fractions VI and VII are the hemins a. Fractions I and IV have not been evaluated sufficiently to provide any assignments for these compounds. Spectra of both reduced and oxidized pyridine hemochromogens of most of these fractions have been recorded. In addition the hemins have been converted to their porphyrin derivatives. The porphyrins have been characterized utilizing chromatography, spectral and chemical methods. Particular emphasis has been placed in these investigations on the hemins a.

720. QUANTUM EFFICIENCY DETERMINATIONS ON COMPONENTS OF THE BACTERIAL LUMINESCENT SYSTEM. Milton J. Cormier* and John R. Totter. Oak Ridge Natl. Lab., Biology Div., Oak Ridge, Tenn.

Quantum efficiency determinations (expressed as molecules utilized per quantum emitted) for dodecyl aldehyde, FMN, and DPNH were made using various enzyme preparations from the luminous bacterium, Achromobacter fischeri. For the aldehyde, the lowest value found for the quantum efficiency was 20, indicating that dodecyl aldehyde disappeared during luminescence. For 2 separate experiments, the efficiency values for FMN were about 0.3 at a point where the luminescence reaction was still proceeding at 60% of the initial rate. The FMN experiments indicate that FMN was not destroyed, but rather recycled at least 3 times during the time interval of the experiment. in 1 enzyme preparation, it was found that approximately 0.036% of the DPNH was utilized for light production, whereas the bulk of the compound was oxidized via other pathways. Flavin mononucleotide and the enzyme luciferase were found to be necessary for oxidation of the aldehyde in the luminescence reaction. The quantum efficiency data support a recent proposal by McElroy and Green that the peroxidation of dodecyl aldehyde (or of long-chain aldehydes in general) furnishes the necessary energy for the excitation of FMN, which subsequently leads to light production.

721. SULFUR AMINO ACID METABOLISM IN LIVER NECROSIS. W. E. Cornatzer and Fred Snyder.* Dept. of Biochemistry, Univ. of North Dakota Med. School, Grand Forks.

Acute liver necrosis was produced in male Sprague-Dawley rats utilizing the method of Koch-Weser et al. (Proc. Soc. Exper. Biol. & Med. 79:196, 1952). Control animals were similarly fasted and received a single injection of corn oil instead of bromobenzene. One minute later all animals were injected with S35 methionine. 1, 3, 6 and 24 hr later the animals were killed and the liver divided into 2 samples, one for histological examination and the other for acid soluble free cystine and protein sulfur fractionation. Bromobenzene administration caused a deficiency of free cystine in the liver with a diminution in the size of total liver cystine pool. There was also a decrease in the synthesis of S35 cystine protein. The decrease in the concentration of free cystine occurred before the histological evidence of necrosis appeared. Acute liver necrosis was produced in another group of animals by the injection of carbon tetrachloride. A decrease in the concentration of free cystine was observed in the liver of these animals.

722. METABOLISM OF CHLORINATED NAPHTHALENES. Herbert H. Cornish,* Walter D. Block and Thomas Dambrauskas.* Dept. of Dermatology and Syphilology, Med. School, and the Inst. of Industrial Health, Univ. of Michigan, Ann Arbor.

Metabolism of naphthalene, α-chloronaphthalene, dichloronaphthalene, tetrachloronaphthalene, pentachloronaphthalene, and octachloronaphthalene has been studied. Following the ingestion of 1 gm of compound by each of 3 rabbits, 66% of the naphthalene, 79% of

the α-chloronaphthalene, approximately 100% of the dichloronaphthalene, and 45% of the tetrachloronaphthalene can be accounted for in the urine. The major excretory product in each case is the glucuronide, with lesser amounts excreted as mercapturic acids, ethereal sulfates, and free phenolic compounds. Pentachloronaphthalene and octachloronaphthalene, do not appear to be metabolized in a similar manner since metabolites of these compounds could not be detected in the present study. The ability of the rabbit to metabolize chlorinated naphthalenes appears to be directly related to the degree of chlorination. Naphthalene derivatives containing not more than 4 chlorine atoms (α-chloro-, dichloro-, and tetrachloronaphthalene) appear to follow a similar metabolic pathway. Naphthalenes substituted with 5 or more chlorine atoms (penta- and octachloronaphthalene) do not seem to follow this pattern. The difference might be explained by the inability of the rabbit to hydroxylate, conjugate, and excrete chlorinated naphthalenes containing 5 or more chlorine atoms. This high degree of halogenation may be sufficient to block the ring positions involved in the formation of a 1:2-dihydronaphthalene-1:2 diol type of compound suggested by Corner and Young (Biochem. J. 61:132, 1955) as an intermediate in the hydroxylation of naphthalene and related compounds.

723. EFFECTS OF HORMONES ON CARBOHYDRATE METABOLISM IN THE ALLIGATOR. Roland A. Coulson, Octavia R. Stevenson* and Thomas Hernandez. Dept. of Biochemistry, Louisiana State Univ. School of Medicine, New Orleans.

The use of cold blooded animals in research on carbohydrate metabolism permits one to observe the effects of stimuli for a period of days instead of minutes. With this fact in mind, epinephrine, hydrocortisone, glucagon and purified bovine somatotropin (Armour) were injected into young alligators for various periods of time. Following the injections, bloods and urines were analyzed for glucose, lactate and electrolytes. Epinephrine and glucagon in optimum doses produced a 250-400 mg % rise in blood glucose. This hyperglycemia lasted for about 70 hr. Even after all of the other typical effects of epinephrine were no longer apparent, the blood glucose fell much slower than in alligators receiving an equivalent amount of exogenous glucose. Whereas epinephrine evoked a marked hyperlactemia which lasted for 10-20 hr, glucagon had no effect on lactic acid. Alligators primed with hydrocortisone showed a greatly decreased ability to dispose of the lactic acid produced by epinephrine. The alligator preserved the stores of both liver and muscle glycogen so effectively that 3 wk without food was insufficient to deplete them. After repeated injections of epinephrine for 12 days to fasting alligators, the glycogen stores returned to the control level within 5 days of the last injection. Both somatotropin and hydrocortisone produced a slight hyperglycemia when given singly. When combined they caused diabetes. (Supported in part by PHS Grant No. H-2062-1.)

724. SEPARATION OF MIXTURES ON THE BASIS OF MOLECULAR SIZE. L. C. Craig, T. P. King* and A. Stracher.* Rockefeller Inst. for Med. Research, New York City.

When 2 solutes differing in size diffuse through a cellophane membrane under a concentration gradient there is a difference in the net rate of transfer of each as would be expected but the difference is of a greater magnitude than would be expected from simple diffusion alone. The membrane exerts a selective action which, with the solutes thus far studied, enhances the difference in rate. This selective action differs with the membrane and the solvent but has been found to be very reproducible for a given size of commercial cellophane tubing. A simple experimental arrangement requiring only a few milligrams of solute has been devised which has proven suitable for quantitatively measuring the dialysis rate of a variety of solutes. That the experimental arrangement is satisfactory has been proven by the finding that the transfer rate of a simple nonassociating solute follows first order reaction kinetics. Suitable cellophane membranes have been found for amino acids to proteins of molecular weight in the range of 45,000. Dialysis rates in every case fall in the inverse order of increasing molecular weight. The size is thus by far the major factor in determining the relative rate of dialysis under standard conditions, although certain anomalies ascribable to charge have been noted. Relative differences in size become more important as the molecules approach the limit of size which will pass through the membrane. Other factors bearing on selectivity have been found. A series of membranes permit separations to be made in a manner entirely analogous to counter-current distribution.

725. EXUDATIVE DIATHESIS AND VITAMIN E DEFICIENCY IN TURKEY POULTS: VITAMIN E DEFICIENCY IN TURKEYS. B. G. Creech,* G. L. Feldman,* T. M. Ferguson,* B. L. Reid* and J. R. Couch. Depts. of Poultry Science and Biochemistry and Nutrition, Texas A. and M. College System, College Station.

Symptoms of vitamin E deficiency which consisted of hemorrhaging on inner sides of the thighs and lower portion of the breast muscle, a mild edema and a macrocytic anemia, were produced by maintaining Beltsville small white turkey poults from 4 maternal diet sources on a Torula yeast diet. The sumptoms were prevented by supplementation of the diet with α-tocopheryl acetate (44 mg/kg) and by a carry-over effect of vitamin E or dried brewers yeast from the dam through the egg to the poult. Electrophoretic studies were carried out on blood serum from these poults and from day-old poults hatched from dams fed practical and synthetic diets alone and supplemented with α-tocopheryl acetate and sources of unidentified growth factors. The apparatus was of the hanging strip paper type with "Durrum" cell (J. Am. Chem. Soc. 72:2943, 1950). Separation was effected on strips of Whatman No. 3MM filter paper using a barbiturate buffer of ionic strength 0.075 and pH 8.3. Running time was 6 hr at a constant current of 15 ma. These studies showed no difference in protein components present or in A/G ratios. Poults hatched from dams fed both the practical and synthetic basal diets showed reduced A/G ratios. The A/G ratio was significantly increased by supplementation of the maternal practical diet with α-tocopheryl acetate and/or dried brewers yeast.

726. TERMINAL GROUPS OF RIBONUCLEIC ACIDS FROM YEAST. Arthur M. Crestfield* and Frank Worthington Allen. Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

Four nucleosides and four diphosphonucleosides have been isolated from alkaline hydrolysates of the ribonucleic acids prepared by Crestfield, Smith and Allen (J. Biol. Chem. 216:185, 1955). Quantitative estimations in percentages of total residues are: adenosine, 0.2, guanosine, 0.07, uridine, 0.1, cytidine,

0.08, diphosphoadenosine, 0.3, diphosphoguanosine, 0.2, diphosphouridine, 0.1, and diphosphocytidine, 0.03. These 8 substances originate 1) from terminal nucleosides and 2) from terminal 5' nucleotides, each esterified to 3' nucleotide residues and represent terminal groups not detected previously in ribonucleic acids from yeast. Together with earlier results this work shows that certain of the ribonucleic acids of yeast have a 3'-terminal and others a 5'-terminal phosphate groups. Hydrolyses were carried out by 1 molar aqueous solutions of piperidine or diethylamine at 55° for 48 hr. The volatile bases were removed by lyophilization, thus eliminating salt removal problems. Dephosphorylation was less than 0.04%. Electrophoretic separation of 5-10 mg portions of the hydrolysate into bands of 1) nucleosides, 2) nucleotides and 3) diphosphonucleosides was achieved at pH 5.7 in ammonium acetate buffer. The nucleosides were separated by chromatography in n-butanol-water (86:14) and by electrophoresis at pH 2. The diphosphonucleosides were resolved by chromatography in isobutyric acid, 0.5 M ammonia (10:6) and by electrophoresis at pH 3.5.

727. STUDY OF THE CITRIC ACID CYCLE IN ERYTHROCYTES. Rashid Dajani* and James M. Orten. Dept. of Physiological Chemistry, Wayne State Univ. College of Medicine, Detroit, Mich.

Earlier work in this and in other laboratories has demonstrated that porphyrins, purines, and perhaps other substances are synthesized from simple precursors in the nucleated erythrocyte. Shemin and his coworkers have shown that the citric acid cycle is involved in the erythrocyte biosynthetic mechanism and have postulated a supplementary "succinate-glycine" cycle. Methods developed in this laboratory have made it possible to separate chromatographically (on silica gel) and quantitatively determine the amounts and specific activities of the individual citric acid cycle acids formed from labeled precursors. In the present study, C^{14}-labeled glycine and acetate were incubated with either nucleated or nonnucleated erythrocytes and the cycle acids were separated and determined. The results conclusively demonstrated the incorporation of both glycine and acetate in the individual cycle acids. However, there were differences between the amounts and specific activities of the cycle acids when either glycine or acetate was used as the precursor. Also, there were differences in the degrees of incorporation of glycine and acetate. The nature and significance of these differences will be discussed. The addition of fluoroacetate increased the amount and C^{14}-activity of the citrate fraction. The data thus conclusively demonstrate biosynthetic mechanism in the nucleated erythrocyte involving the citric acid cycle and that glycine contributes both directly and indirectly to these biosynthetic pathways. Results with nonnucleated erythrocytes will also be discussed. (Aided by grant no. C-2144, Natl. Insts. of Health.)

728. INHIBITION BY CHLORIDE ION OF THE ACTIVATION OF BOVINE PLASMINOGEN (PROFIBRINOLYSIN). Arthur Dalby* and Edwin T. Mertz. Dept. of Biochemistry, Purdue Univ., Lafayette, Ind.

Attempts to purify a crude plasminogen preparation obtained by 30% saturation of bovine serum with ammonium sulfate resulted in an inability to activate the proenzyme with either chloroform or human globulin-streptokinase mixture of Mullertz (Biochem. J. 61:1955). This phenomenon was traced to the isotonic saline used to redissolve precipitates obtained during fractionation. Crude plasminogen preparations dialyzed against 0.01 M potassium phosphates, pH 7.4, are activated to a maximum in 1 hr at 25°C using 1% by volume of the Mullertz mixture. A concentration of 0.05 M sodium chloride completely inhibits this activation, and increasing the concentration of the activator 10-fold has no effect. The inhibitor is the chlorde ion; other anions, inorganic and organic, show lower degrees of inhibition down to acetate ion, which shows no effect. Cations tested to date do not appear to be inhibitory; calcium ions, and, to a lesser extent, magnesium ions, have an activating (or stabilizing) effect. Chloroform activation of crude plasminogen is also inhibited by chloride ions. The length of the lag phase preceding the onset of chloroform-induced activation bears a simple mathematical relationship to the chloride ion concentration. Dialyzed crude plasminogen activates spontaneously in 0.01 M potassium phosphates, pH 7.4; this reaction is completely inhibited by chloride ions. Plasmin activity was measured with a manometric TAMe method (Mertz and Dalby, J. Michigan M. Soc. 55:967, 1956) and a fibrin lysis method (Guest et al. Am. J. Physiol. 150:661, 1947). (Supported by a grant from the Surgeon General's Office, Dept. of the Army.)

729. CONVERSION OF TRITIATED LATHOSTEROL TO CHOLESTEROL BY CELL-FREE PREPARATIONS OF RAT LIVER. Ann G. Davidson,* Elinor G. Dulit* and Ivan D. Frantz, Jr. Cardiovascular Research Lab., Dept. of Medicine, Med. School, Univ. of Minnesota, Minneapolis.

Tritiated lathosterol having a specific activity of 60 μc/mg was prepared by hydrogenation of Δ^7-dehydrocholesterol. The solvent for the hydrogenation was ethyl acetate, and the catalyst Raney nickel. Homogenates were prepared by the method of Bucher (J. Biol. Chem. 206:471, 1954), with the modification that for this reaction, DPN and nicotinamide could be omitted. Two-and-a-half to 10 μg of lathosterol dissolved in 25-100 μl of propylene glycol were added to 2.5 ml of homogenate. After incubation for 1 hr at 37° in an atmosphere of oxygen, the sterols were isolated as previously described (J. Biol. Chem. 206: 465, 1954), passed through the dibromide in some instances, and chromatographed (Frantz et al. J. Biol. Chem. In press). From 3 to 14% of the radioactivity added to the homogenate was recovered in a peak coincident with the cholesterol peak. When the incubation was carried out at 0° in oxygen or at 37° in nitrogen, no radioactivity was demonstrable in cholesterol. We have also demonstrated conversion by a particle-free preparation. A homogenate of rat liver was centrifuged for 90 min at 105,000 times gravity. Ten milliliters of the supernatant were incubated for 1 hr in oxygen with 10 μg of tritiated lathosterol containing 31,000 CPM. On chromatography of the extract, 570 CPM were recovered in a peak coincident with cholesterol.

730. POTENTIALITIES OF ELECTROPHORESIS IN PLASTIC FOAM ARRANGED IN CONTIGUOUS SEGMENTS. Harold M. Davidson (intr. by Siegfried J. Thannhauser). Cancer Research and Cancer Control Unit of the Dept. of Surgery, Tufts Univ. School of Medicine, Boston, Mass.

A spongy polyurethane foam was tested for its applicability in zone electrophoresis of proteins and in other techniques involving the separation of ampholytes in an electric field. Precut pieces of foam were filled with buffer or protein before being assembled in the electrophoretic cell. At the end of the run the contents of the segments in the path of migration were isolated individually by compressing

each sponge separately. The system is adaptable both for zone electrophoresis and for studies on migrations in which gradients (e.g., pH, density) can be superimposed on the electric field. This system has been used both as a preparative and as an investigative tool in enzyme purification, particularly with prostatic acid phosphatase and urinary β-glucuronidase. The advantages of the sponge system are, due to the type and permanency of the matrix, the ease in recovering protein uncontaminated by matrix, its versatility for both macro and ultramicro preparative procedures, and the provision of a new technical approach to the practice of electrophoresis in multi-gradient systems.

731. ACTIVATION OF AMINO ACIDS IN PLANT AND ANIMAL TISSUES. J. Wendell Davis,* Audrey N. Best* and G. David Novelli. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

In a continuing investigation of the activation of amino acids as related to protein synthesis, the amount of amino acid activating system, as measured by PP^{32}-ATP exchange, has been determined as a function of time during the greening of etiolated plants. When the activating system is compared with the incorporation of C^{14} leucine into plant protein, it is found that both systems are present and maximally active in etiolated tissue and do not appear to change significantly in amount during the greening process. The rate of uptake of C^{14} leucine by plant tissue is nearly the same for chloroplasts, grana and mitochondria, but microsomes have a higher initial rate followed by a fall in rate after about 30 min. The amino acid dependent exchange of inorganic pyrophosphate with ATP has been examined in a number of organs of the rat and rabbit. In general, liver has the highest activity, followed by kidney, heart, and spleen. When the activity of individual amino acids in this exchange system in both plant and animal tissues is measured, a group of about 10 amino acids are found to exhibit the greatest activity, while the remaining 12 amino acids show little or no activity. The active group of amino acids are the same as those previously reported for bacteria by DeMoss and Novelli (Biochem. Biophys. Acta 10:592). The significance of this finding will be discussed.

732. BIOSYNTHESIS AND METABOLISM OF L-GULONOLACTONE. Peter G. Dayton,* Carole Evans,* Julian Kanfer* and J. J. Burns. Natl. Insts. of Health, Bethesda, Md., and New York Univ. Research Service, Goldwater Mem. Hosp., New York City.

Previous studies have shown that L-gulonolactone is converted to L-ascorbic acid in rats but not in guinea pigs (Science 124:1148, 1956). Experiments carried out in both species with carboxyl and uniformly labeled L-gulonolactone show that the compound was extensively oxidized in vivo -- 65% to CO_2 within 24 hr. Most of the remaining C^{14} was recovered in urine as L-gulonic acid. The conversion of D-glucurono-lactone-6-C^{14} and D-glucose-1-C^{14} to L-gulonolactone was studied by measuring the incorporation of C^{14} into L-gulonic acid isolated from urine by a specific carrier dilution technique. Following administration of labeled D-glucuronolactone to rats and guinea pigs about 2% of the dose was recovered as L-gulonic acid. Labeled glucose was converted to L-gulonic acid to the extent of about 0.3% in rats receiving either barbital or Chloretone. However, no conversion of D-glucose to L-gulonic acid was detected in nondrug treated rats (< 0.03%). A similar pattern of incorporation of labeled D-glucose into D-glucuronic acid and L-ascorbic acid isolated from urine of normal and barbital-treated rats was also observed. The possibility of an alternate pathway of glucose metabolism in drug-treated rats via L-gulonolactone will be discussed.

733. ENZYMATIC HYDROLYSIS OF THE THIOL ESTER BOND OF β-HYDROXY-β-METHYL-GLUTARYL COENZYME A (HMG CoA). Eugene E. Dekker (intr. by Adam A. Christman). Dept. of Biological Chemistry, Med. School, Univ. of Michigan, Ann Arbor.

Previous studies by Bachhawat, Robinson and Coon (J. Biol. Chem. 216:727, 1955) demonstrated that enzyme preparations from animal tissues catalyze the carboxylation of β-hydroxyisovaleryl CoA to furnish HMG CoA and that the latter compound is rapidly converted to equimolar amounts of acetoacetate and acetyl CoA by the "HMG CoA cleavage enzyme." A different enzyme has recently been found which catalyzes the hydrolysis of the thiol ester bond of HMG CoA with the release of free CoA. The liberation of CoA sulfhydryl groups can be measured colorimetrically when this "HMG CoA deacylase" is incubated at pH 6.4 with synthetically prepared HMG CoA. As demonstrated by this method, the enzyme is present in extracts of liver, kieney, and brain, but not in extracts of pig heart and yeast. The partially purified deacylase from potassium chloride-ethanol extracts of pig kidney contains no HMG CoA cleavage enzyme, and the latter enzyme purified about 15-fold from beef liver extracts has no detectable deacylase activity. Although the function which HMG CoA deacylase performs in animal tissues is not yet clear, by coupling with the cleavage enzyme it might effect the net conversion of acetyl CoA to HMG as follows: acetyl CoA + acetoacetate $\rightleftharpoons$ HMG CoA $\longrightarrow$ HMG + CoA. This proposed mechanism for the reversal of the cleavage reaction could, therefore, account for the incorporation of radioactive acetate into HMG as demonstrated in other laboratories by investigators studying cholesterol biosynthesis.

734. ENZYMATIC SYNTHESIS OF S-ADENOSYL-HOMOCYSTEINE FROM ADENOSINE AND L-HOMOCYSTEINE. G. de la Haba* and G. L. Cantoni. Lab. of Cellular Pharmacology, Natl. Inst. of Mental Health, Bethesda, Md.

S-adenosylhomocysteine (ASR) has been identified previously as the product of enzymatic transmethylations from S-adenosylmethionine (Cantoni and Scarano, J. Am. Chem. Soc. 76:4744, 1954). It has now been shown that ASR may also be formed by the condensation of adenosine and L-homocysteine. The enzyme catalyzing this reaction has been purified 80-fold from rat liver. The reaction is reversible with the equilibrium greatly in favor of synthesis; in fact, appreciable hydrolysis is obtained only when the system is coupled to both adenosine deaminase and thetin-homocysteine methylpherase (Durell and Anderson, Fed. Proc. 15:245, 1956). The purified enzyme is strictly specific for adenosine, with inosine, 3-adenylic acid and 2-desoxyadenosine inactive as substrates. Likewise, of many mercaptans tested only L-homocysteine is utilized as a substrate. S-adenosylhomocysteine has been identified as the product of the reaction by paper chromatography and by chemical methylation to yield a product identical with S-adenosylmethionine as determined 1) by electrophoretic mobility, 2) by paper chromatography, and 3) enzymatically in the methylation of guanidino acetic acid to yield creatine.

735. CHLOROPLAST RESPIRATION OF GLYCOLATE AND ACETATE. L. A. Delavan* and A. A. Benson. Dept. of Agricultural and Biological Chemistry, Pennsylvania State Univ., University Park.

The respiration of phosphorylated intermediates of photosynthesis by concurrent respiration via the Krebs cycle is slow, especially at high light intensities. Brown's (Am. J. Botany 40:719, 1953) results demonstrating constancy of light and dark respiration in a variety of plants suggests a shift in substrate and oxidative pathway for the illuminated chloroplast. An attempt has been made to estimate the relative rates of acetate and glycolate oxidation by isolated spinach (Tetragonia expansa) chloroplasts. Acetate-1-C^{14} was only slowly respired by illuminated chloroplasts whereas it readily yielded $C^{14}O_2$ when fed to whole Scenedesmus cells. Except for a marked "gush" of $C^{14}O_2$ upon illumination its rate of oxidation by Scenedesmus was independent of illumination. Isolated chloroplasts readily metabolized glycolate-1-C^{14} via glycolic acid oxidase. Illumination of chloroplasts in glycolate-1-C^{14} elicited a $C^{14}O_2$ "gush" followed by enhanced $C^{14}O_2$ evolution. Incorporation into metabolic intermediates was also observed. In normal photosynthesis the glycolyl substrates appear to be provided by the very active transketolase metabolism. (Aided by Atomic Energy Comm., Contract AT(30-1) 1876.)

736. IMMUNOCHEMICAL PROPERTIES OF SYNTHETIC POLYPEPTIDES AND POLYPEPTIDYL PROTEINS. Eva Dellert,* Dorothy J. Buchanan* and Mark A. Stahmann. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Studies of the antigenic properties of synthetic polypeptides and polypeptidyl proteins (Stahmann, Tsuyuki, Weinke, Lapresle and Grabar, Compt. rend. 241:1528, 1955) were continued. Antisera from rabbits which were immunized against polypeptidyl heterologous or homologous plasma albumins or synthetic polypeptides of leucine, phenylalanine, lysine or glutamic acid were studied by means of quantitative precipitin determinations. The antibody levels of the sera against the polypeptidyl proteins were determined by precipitating with the corresponding polypeptidyl bovine or rabbit albumins and the unmodified bovine albumin. The antibody levels against the polypeptidyl bovine albumins were greater than the sum of those against the correspondingly modified rabbit albumin and the unmodified bovine albumin. Antisera against the totally synthetic polypeptides did not precipitate the synthetic polypeptides but like the antisera against the polypeptidyl proteins did give precipitates as indicated by ring tests with several purified protein preparations and polypeptidyl proteins. Inhibition of these precipitation reactions by the synthetic polypeptides could not be demonstrated. Nonfatal anaphylactic shock was observed in guinea pigs with the synthetic polypeptides. The polypeptides did not protect against fatal anaphylactic shock induced by subsequent injections of proteins. The results indicate that these synthetic polypeptides are antigenic in rabbits even though the antisera do not give precipitates with the synthetic antigens. Their antigenicity is increased when the polypeptides are attached to either homologous or heterologous albumins.

737. BIOSYNTHESIS OF RICININE. Adelaide M. Delluva,* Ulrich Schiedt* and Samuel Gurin. Dept. Biochem., Univ. of Pennsylvania, Philadelphia, and Max-Planck-Institut fur Biochemie, Munich, Germany.

A convenient method of isolation of ricinine in micro quantities from Ricinus communis L. has been developed. Preliminary studies with C^{14}-labeled precursors have indicated that formate-C^{14}, carbonate-C^{14}, acetate-1-C^{14}, acetate-2-C^{14}, glutamate-1-C^{14}, glutamate-2-C^{14}, and aspartate-3-C^{14} are readily incorporated by the plant in the synthesis of the alkaloid molecule. Formate is most efficiently utilized while aspartate appears to be superior to a number of other amino acids with respect to its incorporation. For these studies, etiolated seedlings were used. The seeds were germinated and, at the time of maximal alkaloid synthesis, the prevursors were either injected into the endosperm or added to the nutrient medium. Following isolation and recrystallization of the alkaloid the material was chemically degraded and the distribution of the isotope determined.

738. VITAMIN D AND CITRATE OXIDATION IN RAT KIDNEY MITOCHONDRIA. H. F. DeLuca,* F. C. Gran* and H. Steenbock. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Since it is well established that vitamin D increases the citrate content of urine and certain tissues, the mechanism of these increases was investigated. Earlier work revealed that additions of vitamin D to nonrachitogenic, as well as to rachitogenic diets, reduced the oxidation of citrate to α-ketoglutarate in homogenates of kidney but not of liver. Cell fractionation techniques have now been used to determine the locality of this effect, and to investigate a possible relation of vitamin D to oxidative phosphorylation. Vitamin D additions to a rachitogenic and a nonrachitogenic diet reduced the oxidation of citrate by kidney mitochondria. This effect was greatly enhanced by a phosphate acceptor. A similar reduction was noted with d-isocitrate, but little or no effect with α-ketoglutarate, glutamate, succinate, pyruvate plus oxalacetate, or with β-hydroxybutyrate. Inasmuch as the efficiency of phosphorylation coupled to the above oxidations was unaffected by vitamin D, it appears that the reduction in citrate and d-isocitrate oxidation was not related to a more efficient coupling of phosphorylation. Also, it was evident that the reduction of citrate oxidation was not due to Ca since kidney mitochondria prepared in isotonic sucrose containing 0.001 M sodium versenate gave similar results to those obtained with mitochondria prepared in isotonic sucrose alone. In contrast to kidney mitochondria, with liver mitochondria, dietary additions of vitamin D had no effect on either citrate or α-ketoglutarate oxidation. These results harmonize with our earlier failure to demonstrate an increase in citrate content of liver when vitamin D was fed.

739. LOCALIZATION OF A FATTY ACID-SYNTHESIZING SYSTEM IN PIG LIVER. Miriam A. Derks and R. O. Moore (intr. by J. B. Brown). Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

The biosynthesis of long chain fatty acids by pigeon liver preparations has been demonstrated by Wakil et al. (Fed. Proc., 1956). A similar preparation has been obtained from pig liver by centrifugation at 144,000 × g for 15 min to remove higher-density particles. Fatty acid formation from acetate by this preparation resembles that of pigeon liver in its requirement for ATP, Mg^{++}, Mn^{++}, DPNH, TPNH, CoA, glucose-1-phosphate, isocitrate, and phosphate-bicarbonate buffer (pH 8.0). In addition, K^+ and glycerophosphate enhance incorporation of acetate-1-C^{14} into fatty acids. UTP and GTP also increase

long chain fat acid biosynthesis in this system. In contrast to UTP and GTP, CTP decreases acetate incorporation into long chain fatty acids. A significant portion of the fatty acid synthesizing activity is associated with a fraction sedimented by further centrifugation at 144,000 × g for 2 1/2 hr.

740. RELATIONSHIPS OF MACROGLOBULINS TO THEIR MONOMER UNITS. H. F. Deutsch and Jane I. Morton.* Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison.

Recent findings from our laboratory have shown that the addition of sulfhydryl compounds such as cysteine and mercaptoethanol disaggregates serum macroglobulin systems to proteins having molecular weights near 160,000. Different purified macroglobulins show divergent electrokinetic and isoelectric point properties but each gives a single peak on electrophoretic analyses over a wide range of pH. In the ultracentrifuge they are found to consist chiefly of three very concentration dependent components sedimenting at 18, 25, and 32 Svedberg (S) units. The three components exist in nearly the same ratios in different purified preparations, being about 80, 15 and 5%, respectively. The monomer unit of these macroglobulins resulting from the action of mercaptans shows two electrophoretic components both of which have lower isoelectric points than the original molecules. If the sulfhydryl compound is removed somewhat over 50% of the monomer system reaggregates to two concentration dependent components sedimenting at 15.5 and 17.5 S. The dissociated macroglobulins show a marked increase in solubility. Their immunochemical specificities are unaltered in terms of the parent molecule. Quantitative precipitin studies of monomers, reaggregated material, and original macroglobulins reveal changes in combining ratios at the point of maximum precipitation that may be related to the molecular weight variations of the different fractions. These and other physical and immunochemical properties of macroglobulins and their dissociated units will be presented. (Supported by a grant from the Natl. Insts. of Health, PHS.)

741. SOLUBILIZATION OF PYRIDINE-NUCLEOTIDE ENZYMES OF THE GLYCOSIDASE TYPE FROM MAMMALIAN TISSUES. Herbert W. Dickerman and Francis E. Stolzenbach (intr. by Arda A. Green). McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

Mammalian enzymes catalyzing the cleavage of the nicotinamide-ribose bond of DPN, TPN, and nicotinamide mononucleotide (NMN) are found to be associated with the insoluble fractions of etythrocytes, brain and spleen. Studies of the mechanism of these enzymes and their inhibition by nicotinamide have been impeded by the insolubility of existing preparations. A solubilization of the beef spleen DPNase (Alivisatos and Woolley. J. Biol. Chem. 219:823, 1956) of 13% occurred by treatment with isoamyl alcohol and deoxyribonucleic acid. In the present study, solubilization of the brain and erythrocyte stromal DPNase from sheep has been effected by homogenization with equal volume of 5% sodium deoxycholate. Seventy-five per cent of the brain DPNase is solubilized, following which the enzyme has been purified by calcium phosphate gel and ammonium sulfate fractionation. The total purification from the crude homogenate is 280-fold. There is no loss of nicotinamide inhibition at this stage of purification. Physical and chemical properties of the soluble enzyme and the specificity of the enzyme will be presented. The DPNase of sheep erythrocyte stroma has also been solubilized to an extent of 35%. Like the enzyme obtained from pig brain, the soluble enzyme is sensitive to nicotinamide inhibition. Studies of the rates of reaction of the soluble fraction did not indicate the presence of a specific TPNase. The DPNase, TPNase, and NMNase activities of human erythrocyte stroma have all been solubilized. The NMNase has been differentiated from DPNase by its thermal lability. The NMNase activity is not associated with the nicotinamide-ribosidase of the erythrocyte.

742. ISOLATION AND CHARACTERIZATION OF THE BITTER PRINCIPLES IN PEANUT HEARTS. Julius W. Dieckert and Nelle J. Morris (intr. by A. M. Altschul). U. S. Dept. of Agriculture, Southern Regional Research Lab., New Orleans, La.

As part of a program on peanut flavors, a mixture of bitter saponins was isolated from peanut hearts according to the following procedure. The hearts were extracted with 80% alcohol, an aqueous suspension of the alcohol extractables defatted with ether, and the bitter materials adsorbed from the aqueous phase on charcoal. After elution with 50% alcohol, the bitter principles were precipitated from aqueous solution with dilute HCl. The precipitate was washed with dilute HCl and water, dissolved in alcohol, and decolorized with charcoal. Eight kilograms of hearts yielded 4.5 gm of amorphous bitter material containing C, H, and O, but no N, P, or S. Chromatography on glass paper impregnated with silicic acid showed no free sugars. The preparation frothed when shaken with water, gave a red color with H_2SO_4 and gave a positive Molisch test. These properties suggested that the substances were saponins. Chromatography on glass paper impregnated with phosphate buffer showed two materials giving a red color with H_2SO_4. For preparative purposes a column version of the glass paper technique was developed. Using chromatography on glass paper impregnated with silicic acid, three aglycones were found among the products of acid hydrolysis of the bitter principles. Two of the aglycones were obtained in crystalline form by chromatography on a silicic acid column. Melting points, data from chromatography, and behavior toward H_2SO_4 suggested that the crystalline substances were soya sapogenols A and C. Thus, it appears that the bitter principles in peanut hearts are saponins similar to those found in soybeans, clover, and alfalfa.

743. URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES BY PATIENTS WITH RHEUMATOID ARTHRITIS. Nicola Di Ferrante (intr. by D. D. Dziewiatkowski). Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Urinary excretion of acid mucopolysaccharides was measured in 6 patients with active, untreated rheumatoid arthritis. The method described by Di Ferrante and Rich (J. Lab. & Clin. Med. 48:491, 1956) was used, and the results were expressed as mg of glucuronic acid/24 hr. It was found that male patients excreted daily the equivalent of 8.0-12.0 mg of glucuronic acid, as compared with an average normal value of 6.0 mg for individuals of the same age. Female patients excreted daily the equivalent of 5.8-7.4 mg of glucuronic acid, as compared with an average normal value of 3.7 mg. In 4 patients the influence of oral treatment with sodium salicylate (2.4-4.5 gm/day) on the urinary excretion of acid mucopolysaccharides was studied. It was found that under such a treatment, the excretion of acid mucopolysaccharides immediately decreases, approaching normal values. Addition of known amounts of chondroitinsulfate to

urine of patients under salicylate treatment resulted in a recovery of 94 - 104%, thus ruling out the possibility that products of excretion of sodium salicylate could interfere with the reaction between acid mucopolysaccharides and the precipitant. The analytical data of the acid mucopolysaccharides excreted by patients with rheumatoid arthritis will be discussed. (Supported by the Atomic Energy Comm.)

744. GLUCOSE CATABOLISM PATHWAYS IN RAT BRAIN. David DiPietro* and Sidney Weinhouse. Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.

Various glycolytic and respiratory properties of rat brain preparations have been studied with the aid of C^{14} labeling. In slices incubated in saline media under oxygen, lactic acid accumulates rapidly; then, depending on the initial glucose level, either increases slowly or declines. In contrast, oxygen consumption and glucose oxidation continue steadily at linear rates for at least 4 hr. More than 90-95% of the glucose disappearing was accounted for by lactate and CO_2. The proportion of glucose carbon 1 in lactic acid was almost exactly 1/6 of all glucose carbons. After short incubation periods the proportion of C1 in the CO_2 was very low, but gradually approached the theoretical value of 1/6 with longer incubation. Glucose oxidation largely supplemented rather than replaced the endogenous respiration; even at glucose concentrations of 0.05 molar, 30-40% of the respiration was due to endogenous substrates. Whole, isotonic homogenates oxidized glucose readily, but in contrast with similar preparations of other tissues, required added ATP. The pattern of respiration in homogenates was essentially similar to that of slices. Although the oxidative pathway appeared nonoperative, as in slices, preferential oxidation of glucose carbon 1 could be stimulated by addition of TPN+.

745. EQUILIBRIUM BETWEEN GLUCOSE- AND FRUCTOSE-6-PHOSPHATE, AND SEDOHEPTULOSE PHOSPHATE, TRIOSE PHOSPHATE AND PENTOSE PHOSPHATE IN HUMAN HEMOLYSATES. Zacharias Dische. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.

Glucose-6-phosphate (G6P) added to dialyzed fluoridated hemolysate, adjusted to pH 8.4, is partly converted to F6P and a mixture of sedoheptulose phosphate (SdP) identified after dephosphorylation by paper chromatography with 3 solvents, ribose- and ketopentose-5-phosphate, triose phosphate (TrP), and small amounts of tetrose phosphate. After 5-1/2 hr at 33° an equilibrium is reached. Hexose phosphate which disappears, determined enzymatically, is accounted for by the reaction products determined by specific color reactions. After inactivation of transaldolase by incubation of the hemolysate at pH 8.4, at 4° for 4 hr before addition of G6P, conversion of G6P takes place only if TrP is also added. Larger amounts of tetrose phosphate now appear and increase with increase of added TrP. Relative concentrations of SdP, TrP, and pentose-5-phosphate, in equilibrium with G6P in fresh hemolysates, correspond to the relative concentrations of these esters found in equilibrium when ribose-5-phosphate (R5P) is added to the hemolysate after inactivation of transaldolase. Conversion of G6P to the other esters leads, therefore, to a reversible equilibrium between G6P, F6P, and the other esters. In fresh hemolysates, initial presence of TrP is not necessary for conversion of G6P, as G1P which is converted to G6P in hemolysates produces the reaction products and equilibrium as G6P even if possible contamination by catalytic amounts of TrP or FDP is excluded by heating of G1P before addition for 30 min in 0.2N NaOH at 100°.

746. ACYLATION OF THE ENZYMATIC SITES OF CHYMOTRYPSIN AND TRYPSIN. Gordon H. Dixon* and Hans Neurath. Dept. of Biochemistry, Univ. of Washington, Seattle.

δ-Chymotrypsin and trypsin each react stoichiometrically with a number of acylating agents, yielding, at pH 5-6, stable inactive monoacyl enzymes, which at higher pH (7-8) hydrolyze into active enzyme and the acyl ion. Acylation of δ-chymotrypsin has been achieved not only with the ester, p-nitrophenyl-acetate, but also with a series of homologous esters, acid anhydrides and acid chlorides, including acetic, propionic, butyric, valeric, hexanoic and heptanoic anhydride, benzyl and phenylacetyl chloride, as well as the ester, carbobenzoxyglycyl-p-nitrophenolate. The kinetics of acylation has been followed spectrophotometrically, and the kinetics of deacylation of chymotrypsin by measuring the increase in the rate of hydrolysis of acetyl tyrosine ethyl ester when acyl chymotrypsin was incubated with this substrate. While the rate of acetylation of chymotrypsin by p-nitrophenylacetate is about 40 times faster than that of trypsin, both enzymes are deacetylated at comparable rates. With both cymotrypsin and trypsin the pH dependence of both acylation and deacylation can be described by the dissociation curve of a single group having a K_i at 25° of 6.0×10^{-7} for acylation, and 1.1×10^{-7} for deacylation. It is concluded that, while it is likely that serine is the receptor of the acyl group, the participation of an ionizing group is required for both processes. The reactivity of the acyl group is abolished by denaturation of the protein by 8 molar urea. However, the group is retained on the protein and, when denaturation is reversed, it becomes fully reactive again. The significance of these findings in relation to the mechanism of catalysis by chymotrypsin and trypsin will be discussed.

747. ROLE OF VITAMINS IN NEOGENESIS OF METHYL GROUP OF METHIONINE IN VITRO. V. M. Doctor* and J. Awapara. Univ. of Texas, M. D. Anderson Hosp. and Tumor Inst., Houston.

Preliminary studies have been conducted on the role of folic acid (PGA), pyridoxal phosphate and vitamin B_{12} in the incorporation of serine-3-C^{14} and formaldehyde-C^{14} into methyl group of methionine by freshly prepared extracts of chick liver acetone powder. The incubation system consisted of an enzyme preparation, one of the two C^{14} compounds listed above, homocysteine, $MgCl_2$, DPN and ATP. Addition of PGA or tetrahydro PGA to the above system enhanced the incorporation of serine-3-C^{14} or formaldehyde-C^{14} into methionine. The stimulatory effect by folic acid in the above incorporation was blocked by aminopterin while the latter did not influence the augmentation by tetrahydro PGA. Vitamin B_{12} does not affect the incorporation of serine-3-C^{14} or formaldehyde-C^{14} into methionine, while pyridoxal phosphate inhibits the incorporation of serine-3-C^{14} into methionine and enhanced significantly the incorporation of serine-3-C^{14} into alanine. (Supported by PHS Grant C-3047.)

748. FRUCTOSE AND ERYTHROSE METABOLISM IN A STRAIN OF ALCALIGENES FAECALIS. Goetz F. Domagk (intr. by E. O. Keiles). Natl. Insts. of Health, Bethesda, Md.

A strain of Alcaligenes faecalis able to grow on D-erythrose has been isolated by enrichment culture (Hiatt and Horecker. J. Bact. 71:649, 1956). It has

now been shown that suspensions of fructose-grown Alicaligenes faecalis (ATCC #4741) will oxidize erythrose nearly as well as fructose, whereas glucose-grown cells show very little activity with erythrose. Sonic extracts of fructose-grown cells contain a fructokinase which has been purified about 60-fold by a procedure including protamine precipitation, adsorption on calcium phosphate gel and fractionation with ammonium sulfate. The final preparations are stable in the frozen state. The reaction product has been isolated and identified as fructose 6-phosphate; fructose 1-phosphate is not an intermediate in the reaction. The purified preparations still show glucokinase activity but there is evidence that this enzyme is distinct from the fructokinase. The K_m value for fructose is about 10^{-3} M/l. and Mg^{++} is required. The finding that fructose-grown cells will metabolize erythrose and contain an adaptive fructokinase is consistent with the following scheme suggested by Hiatt and Horecker: 1) fructose + ATP $\xrightarrow{\text{fructokinase}}$ fructose 6-phosphate + ADP, 2) fructose 6-phosphate + erythrose $\xrightarrow{\text{transketolase}}$ fructose + erythrose 4-phosphate. Extracts of fructose-grown cells contain a very active transketolase and evidence for reaction 2 has been obtained with these extracts. The purification of transketolase from this source is in progress.

749. METABOLISM OF QUERCETIN BY RAT KIDNEY PREPARATIONS. Carl D. Douglass and Rose Hogan (intr. by Paul L. Day). Dept. of Biochemistry, Univ. of Arkansas School of Medicine, Little Rock.

The metabolism of quercetin, 3,5,7,3',4'-pentahydroxyflavone, added to homogenates of rat kidney has been studied by noting the rate of decrease in optical density at 375 mμ (an absorption maximum of the compound). These experiments in vitro have revealed a rapid degradation of this flavonoid pigment by the tissue. The effects of a number of variables on the process have been investigated. The optimal pH has been found to be near neutrality. Of a variety of common enzyme inhibitors which were tested, only cyanide was inhibitory at 0.001 M. Partial purification of the enzyme responsible for the disappearance of the 375 mμ band has been accomplished by conventional methods. A paper chromatographic survey of extracts prepared from incubation mixtures consisting of quercetin and either whole homogenates or mitochondria and which were treated with various enzyme poinsons has been carried out. This has revealed the accumulation of hitherto unreported intermediates in the presence of fluoride and p-chloromercuribenzoate. Properties of these compounds will be reported. Manometric studies have failed to demonstrate the participation of oxygen in the metabolism of quercetin. (Supported by a research grant from the Natl. Vitamin Fndn.)

750. SPECIFIC LESION OF RENAL SPILLAGE OF PLASMA ALBUMIN IN EXPERIMENTAL NEPHROSIS. David L. Drabkin and Julian B. Marsh. Dept. of Biochemistry, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

When determined by a quantitative precipitin reaction with rabbit anti-serum, prepared against purified rat plasma albumin, the proteinuria of the experimentally nephrotic rat can be shown to be practically exclusively due to plasma albumin. The damaged glomerular basement membrane presumably remains impermeable to the major plasma globulins. Hence, it was of interest to test the degree of urinary spillage of proteins other than plasma albumin in this experimental disease state. It was particularly cogent to study the excretion of intravenously injected rat hemoglobin, since this chromoprotein and plasma albumin are of similar molecular size, and since extra-cellular hemoglobin, beyond certain plasma levels, is not totally retained by the normal glomerular apparatus (Drabkin, Physiol. Rev. 31:345, 1951). Under comparable conditions of injection (about 0.002 mM or 0.033 gm of hemoglobin/0.3 ml in 200 gm rats), the albuminuria of nephrotic rats remained unaltered in amount, but the urinary spillage of hemoglobin was at essentially the same level as that in normal control animals - of the order of 30% of the injected chromoprotein in each group. Injected rat and bovine plasma albumins were excreted to the same degree by the nephrotic animals. The findings suggest that the tissue lesion is specific for plasma albumin.

751. CATALYTIC ACTIVITY OF CARBOXYPEPTIDASE-DEGRADED ALDOLASE. Edward R. Drechsler (intr. by M. O. Schultze). Depts. of Agricultural Biochemistry and Physiological Chemistry, Univ. of Minnesota, Minneapolis.

Crystalline fructosediphosphatealdoase from rabbitmuscle (Taylor et al. J. Biol. Chem. 173:591, 1948) underwent a limited degradation by crystalline carboxypeptidase at pH 7.7 and 30° to a product retaining approximately 7% of the original catalytic activity toward fructose-1,6-diphosphate. Treatment of such solutions with more carboxypeptidase did not reduce this residual activity, indicating that a new molecular species of enzyme had been formed. This modified enzyme was crystallized from ammonium sulfate solution and found to be indistinguishable from the original crystalline fructosediphosphatealdolase in the ultracentrifuge. Chemical and paper chromatographic analyses indicated that only tyrosine and probably threonine were liberated in significant amounts. Under suitable conditions, loss of aldolase activity proceeded initially (5 min) very rapidly, at a rate essentially proportional to the rate at which free tyrosine was liberated. Maximum activity loss was reached when 3 M of tyrosine were released per mole of aldolase. These results emphasize the necessity of tyrosine as an integral component of the active fructosediphosphatealdolase. Fructose-1-phosphatealdolase activity (Leuthardt and Wolf. Helvet. physiol. et pharmacol. acta 13:C20, 1955) of the crystalline aldolase used in these experiments was initially very low but increased three-fold upon degradation by carboxypeptidase of the aldolase to the minimum residual activity. This suggests the possibility of a conversion of fructosediphosphate-aldolase to fructose-1-phosphatealdolase by the action of carboxypeptidase.

752. SEPARATION OF CATECHOLAMINES FROM CATECHOL ACIDS BY ALUMINA. William Drell. Dept. of Physiological Chemistry, Univ. of California Med. Ctr. and VA Ctr., Los Angeles.

Catecholamines are frequently concentrated from biological extracts by adsorption on alumina under alkaline conditions. Present studies indicate that catechols are adsorbed quantitatively at pH 6.1 in 0.1 - 0.5 M acetate buffer on alumina columns (8 x 35 mm) in the absence of phosphate ion. After washing with 0.5 M buffer, pH 6.1, the catecholamines (epinephrine, arterenol, dopamine, N-isopropylarterenol) are specifically eluted with water (30 ml.) and 0.01 M acetate buffer, pH 4 (10 ml.). Recoveries are 90%. The catechol acids (catechol acetic acid, dihydroxypenylalanine, dihydroxyphenylserine) are then quantitatively eluted with 0.2 N acetic acid

(30-40 ml). Adrenal slices incubated with C^{14} labeled tyrosine under the conditions of Kirchner and Goodall (Fed. Proc. 15:110, 1956) and fractionated by the described alumina procedure yield a catecholamine fraction of high radioactivity. Subsequent treatment by the cation exchange procedure of Kirchner and Goodall yields results similar to those reported by these workers. The catechol acid fraction contains about a third the amount of radioactivity found in the amine fraction. Incubation of adrenal slices with labeled tyrosine in the presence of the depa decarboxylase inhibitor, 5-(3-hydroxycinnamoyl)-salicylic acid (Hartman, Akawie and Clark, J. Biol. Chem. 216:507, 1955) results in a marked decrease in radioactivity in the catecholamine fraction and an increase in radioactivity in the acid fraction. The nature of this change is under investigation. (Aided by grants from the American and Los Angeles County Heart Assoc.)

753. ENZYMATIC REACTIONS INVOLVING 6,8-DITHIOLOCTANOIC (DIHYDROLIPOIC) ACID AND ITS ACETOACETIC THIOESTER. George I. Drummond* and Joseph R. Stern. Dept. of Pharmacology, Western Reserve Univ., Cleveland, Ohio.

Acetoacetate synthesis by liver fractions from a source of acetyl-S-CoA (generated by transacetylase from acetyl phosphate and CoA-SH) requires the addition of a thiol. Among active thiols, DL-6,8-dithioloctanoic acid (DL-DTO) has the greatest activity, half maximum activation occurring with 1×10^{-4} M DL-DTO compared to 3.5×10^{-4} M dimercaptopropanol and 2×10^{-3} M glutathione. Therefore, some fatty acid thioesters of DL-DTO have been synthesized and tested for enzymatic reactivity with the following results. 1) Synthetic S-acetoacetyl-DL-DTO (?8-ester) is hydrolyzed by enzyme fractions from liver but apparently not from other tissues. The enzyme concerned has been purified 40-fold from chicken liver and shown to be different from acetoacetyl-S-glutathione thioesterase. 2) As measured optically, liver fractions catalyze a disappearance (?deacylation) of acetoacetyl-S-CoA and acetoacetyl-S-pantetheine, provided DL-DTO (or certain monothiols) is added. The products of this reaction have not yet been identified. 3) These fractions, however, do not catalyze thiolysis or acyl transfer reactions between S-acetoacetyl-DL-DTO and DL-DTO or CoA-SH or pantetheine or glutathione. 4) 6-S-acetyl-DL-DTO and 8-S-acetyl-DL-DTO are not converted to acetoacetate by liver fractions which so convert acetyl-S-CoA. 5) S-acetoacetyl-DL-DTO is reduced by DPNH and crystalline heart β-hydroxybutyryl-S-CoA dehydrogenase. During partial purification of the acetoacetate synthesizing system, activity 1 is largely resolved, while activity 2 is concentrated. This suggests activation of acetoacetate synthesis by DL-DTO may not involve S-acetoacetyl-DL-DTO as intermediate. The properties of the above reactions will be presented and possible roles of DL-DTO discussed.

754. EXCHANGE OF HYDROGEN BETWEEN REDUCED PYRIDINE NUCLEOTIDES AND WATER. George R. Drysdale (intr. by Mildred Cohn). Biochemistry Dept., Washington Univ. School of Medicine, St. Louis, Mo.

It has been reported that under conditions where net oxidation does not occur, intact liver mitochondria and the highly purified DPNH- cytochrome -c reductase of heart musle catalyze an exchange reaction between DPNH and D_2O (Drysdale and Cohn. Biochim. et biophys. acta 21:397, 1956). The exchange reaction is stereospecific and produces DPND with the β configuration. Independent evidence that the D was incorporated into position 4 of the nicotinamide ring has been adduced by analysis of the 2 and 6 pyridones of n-methyl nicotinamide obtained from DPND labelled by the exchange reaction (Pullman, San Pietro and Colowick. J. Biol. Chem. 206:129, 1954). It was previously reported that the exchange reaction catalyzed by the DPNH-cytochrome-c reductase was inhibited by low concentrations of p-chloromercuribenzoate, but it has now been found that atabrine at concentrations sufficient to inhibit the overall reaction about 70%, does not inhibit the exchange reaction. The exchange reaction has been examined in liver microsomes and found to show the same stereospecificity as in mitochondria. The conclusion of Vennesland that milk xanthine oxidase preparations did not show stereospecificity towards DPND has been confirmed by the exchange technique. Preliminary studies using tritiated water with partially purified TPNH - nitrate reductase preparations from Neurospora crassa, indicate that addition of FAD to the resolved enzyme stimulates the rate of the exchange reaction much less that it stimulates the rate of the overall reaction. The implications of these results for the mechanism of the hydrogen exchange will be discussed.

755. CHLORIDE TRANSPORT AND ELECTRICAL PHENOMENA IN THE STOMACH. Richard P. Durbin* and Erich Heinz. Biophysical Lab., Harvard Med. School, and Dept. of Biochemistry, Tufts Med. School, Boston, Mass.

Mechanism of chloride transport and its relationship to electrical phenomena and acid secretion in the isolated gastric mucosa of the frog have been studied in further detail. The presence of a large, nonconducting component of the chloride flux through the mucosa has been previously cited by Hogben as evidence for a carrier-exchange system in the stomach. In the present work, it has been found that various kinds of metabolic inhibition, including 2:4 dinitrophenol, cyanide, and anoxia, considerably reduce this component, but do not completely eliminate it. This component, however, was found to be absent in an irreversibly damaged or dead mucosa, as shown by the close agreement between measured conductance and that calculated from the ion fluxes. It is concluded that the mechanism of chloride transport through the gastric mucosa is metabolically controlled. This mechanism, which is probably responsible for the generation of the electrical potential difference, does not seem to be essential for the acid secretion. Further observations with more specific inhibitors, such as Diamox and thiocyanate, seem to support the hypothesis that acid secretion and generation of the potential difference are two separate functions of the gastric mucosa.

756. FREE GLUTAMINE AND GLUTAMIC ACID LEVELS IN THE PRESENCE OR ABSENCE OF AMMONIA IN VIVO. Jean Paul DuRuisseau, Jesse P. Greenstein, Milton Winitz and Sanford M. Birnbaum. Lab. of Biochemistry, National Cancer Inst., Bethesda, Md.

Earlier reports from this Laboratory indicated that although intraperitoneal administration of 1 mm/kg of L-Arginine·HCl or 4 mm/kg of monosodium L-glutamate or L-glutamine alone afforded no appreciable protection in adult male rats against lethal dose of ammonium acetate, their combination resulted in a high degree of protection against the latter. In an attempt to explain this protection, NH_3, urea, aspartic acid, glutamic acid and glutamine

levels were determined in various tissues, i.e., blood, brain, muscle, and liver, both in the presence and absence of a lethal dose of ammonia. When a mixture of 1 mm/kg of L-arginine·HCl and 4 mm/kg of L-glutamine is administered 1 hr prior to a lethal dose of ammonia, the level of free ammonia in the liver is higher than if an equivalent amount of monosodium L-glutamate is used in lieu of L-glutamine. The level of liver urea is, however, identical for both mixtures and well above normal. The level of glutamine in the liver of an ammonia injected rat invariably decreased, whether or not prior protection with the above mixtures had been afforded. Finally, 1 mm/kg of L-arginine·HCl and 4 mm/kg of sodium chloride injected 1 hr prior to an $LD_{99.9}$ dose of ammonium acetate results in a protection of approximately 70%. When the level of free, tissue glutamine is determined prior to the injection of the ammonia, a general increase is found. This evidence is suggestive of an interrelation of the responses shown to the individual arginine-NaCl, the arginine-sodium glutamate and arginine glutamine combinations. Such a working hypothesis will be presented.

757. SOME NEW AUTOTROPHIC HYDROGEN UTILIZING BACTERIA. M. Dworkin* and J. W. Foster. Dept. of Bacteriology, Univ. of Texas, Austin.

Several organisms capable of utilizing ethane as the sole source of energy and carbon were isolated from soil and sewage by means of enrichment cultures consisting of mineral salts and 50% illuminating gas and 50% air. The bacteria included 5 different members of the genus Mycobacterium, one Flavobacterium, one Alkaligenes, one gram-negative coccus, and a filamentous fungus tentatively identified as belonging to the genus Acremonium. Four of the mycobacteria and the gram negative coccus were able to grow extensively in mineral salts solution in a hydrogen-oxygen-carbon dioxide atmosphere. The autotrophic nature of this growth was confirmed by extensive gas analysis and $C^{14}O_2$ uptake experiments. All the organisms could grow on various organic substrates in the absence of hydrogen gas hence, like all known hydrogen bacteria, are facultative autotrophs. Washed cells of the coccus oxidized hydrogen immediately when they were grown on hydrogen; hydrogen oxidation was adaptive with glucose or with ethane grown cells. In the case of Mycobacterium no. 5, hydrogen oxidation was constitutive, i.e., no adaptive lag was observed, regardless of whether the cells were grown on hydrogen, ethane or glucose. With Mycobacterium no. 8, cells grown on hydrogen or ethane oxidized hydrogen immediately, whereas glucose grown cells oxidized hydrogen adaptively. The mycobacteria and the aerobic coccus are the first reported autotrophic representatives of these groups. Additional aspects of the metabolism of these organisms have been studied.

758. EFFECT OF VANADYL SULFATE ON PLASMA AND TISSUE CHOLESTEROL LEVELS AND ATHEROSCLEROSIS IN THE CHICKEN. Charles H. Eades, Jr. and Duane G. Gallo (intr. by Homer E. Stavely). Dept. of Biochemical Research, Mead Johnson Research Labs., Evansville, Ind.

It has been reported that vanadium salts affect cholesterol metabolism in the rat (Curran, J. Biol. Chem. 210:765, 1954) and in the rabbit (Curran and Costello, J. Exptl. Med., 103, 49, 1956) (Mountain, Stockwell and Stockinger, Proc. Soc. Exper. Biol. Med. 92:582, 1956). There are other scattered reports concerning the influence of vanadium compounds on lipid metabolism. It was of interest to study the effect of vanadyl sulfate on plasma and tissue cholesterol levels in the chicken. Day old white leghorn cockerels were fed a high protein ration for 7 wk at which time they were divided into 3 groups: 1) Stock diet, 2) Stock diet + 2% cholesterol, 3) Stock diet + 2% cholesterol + 0.05% vanadyl sulfate. The experimental diets were fed for 8 wk; blood was drawn from wing veins at 0, 2, 5, and 8 wk of the experimental period. Plasma was analyzed for cholesterol and lipid phosphorus. After the experimental 8 wk, the chickens were killed and the aortic atherosclerosis graded. Aorta, heart, and liver were analyzed for cholesterol and lipid phosphorus. The results indicate that vanadyl sulfate inhibits the rise in plasma cholesterol to a significant extent while the plasma lipid phosphorus remains at or near control values. The tissue values followed a similar pattern with the exception of aorta in which there was little difference between groups 2 and 3 with respect to total cholesterol and lipid phosphorus. However, the vanadyl sulfate appeared to reduce the severity of atherosclerosis in the aorta.

759. REDUCTION OF DISULFIDE BONDS IN α CHYMOTRYPSINOGEN. Richard Egan and Eston M. Gross (intr. by B. J. Jandorf). Enzyme Chemistry Branch, Chem. Warfare Labs., Army Chem. Ctr., Md.

Complete reduction of the disulfide bonds in α chymotrypsinogen (Chtgen) by K_2SO_3 can be accomplished in the presence of p-chloromercuribenzoate, an agent which reacts with the SH groups as they are formed. Analysis of the number of S-S bonds reduced was performed as described earlier (Gross and Egan, Fed. Proc. 15:866, 1956). The completely reduced Chtgen was devoid of potential enzymatic activity. Significant structural breakdown is associated with reduction: up to 50% of the material passed through a Visking dialysis membrane and was soluble in 20% trichloroacetic acid; in the ultracentrifuge the total reduction mixture produced a single peak with S_{20} values between 0.5 and 0.7 depending on the concentration. Free diffusion studies indicate a D_{20} of 14×10^{-7} cm^2/sec with a mesokurtic frequency distribution. An average molecular weight of ca. 5,000 may be calculated from these data; however the material obtained in reduction is heterogenous since free electrophoresis at pH 9 and continuous flow paper electrophoresis at pH 9.2 showed the presence of 5 components. Since no additional DNFB-reactive α amino groups were found after complete reduction, it appears that no peptide bonds have been hydrolyzed during the reduction. On this basis, the hypothesis is presented that the disulfide linkages, rather than serving as secondary cross linking groups, are the primary connection between various segments of the Chtgen molecule, and that all but one of these segments should be cyclic peptides. The mechanisms involved in the reduction will also be discussed.

760. METABOLISM OF GLUCURONIC ACID IN VIVO. Frank Eisenberg, Jr., Natl. Insts. of Health, Bethesda, Md.

Whereas glucuronolactone is readily catabolized to CO_2 in the intact rat, glucuronate has been reported to be relatively inert. Since glucuronate is metabolized in vitro, an explanation has been sought for its apparent inertness in vivo. Glucuronate-C^{14} was prepared from glucuronolactone-C^{14}, recrystallized, and shown to be free of glucuronolactone by paper chromatography. Rats were injected intraperitoneally with 333 μM/kg of glucuronate-C^{14} and respiratory CO_2 was collected hourly for 6 hr. Radioactivity was determined by scintillation counting in Hyamine base. Urine was collected for 24 hr, chromatographed on paper, and

the papers were sprayed with naphthoresorcinol. In 16 experiments the incorporation of injected C^{14} into CO_2 varied from 3.5 to 73%. In 10 of the 16 the incorporation was 8% or less; in 3, 30 to 50%; and in 3, above 70%. Where combustion exceeded 70% no naphthoresorcinol-reacting substances could be detected in the urine; all other urine samples contained such material, which is presumed to be glucuronate. In order to ascertain to what extent variations in combustion might reflect variations in renal excretion of glucuronate, as the results above suggest, the metabolism of the compound was studied in nephrectomized rats. Glucuronate-C^{14} was injected intracavally at the time of nephrectomy. In 7 experiments the combustion varied only from 12 to 18% in 6 hr and from 48 to 69% in 24 hr. It is concluded that glucuronate is actively catabolized in vivo and that the wide variations observed are a reflection of its variable excretion by the kidney.

761. PLASMA AND URINARY CORTICOIDS DURING A CYCLE OF MORPHINE ADDICTION. Anna J. Eisenman, H. F. Fraser and J. W. Brooks.* Natl. Inst. of Mental Health Addiction Research Ctr., Lexington, Ky.

Urine and plasma 17-hydroxycorticoids were studied on 2 subjects during a cycle of morphine addiction: control period, 40 days; addiction, 60 days; abrupt withdrawal; recovery, 50 days. ACTH or hydrocortisone was administered at least once during each phase. Placebos were given several times during the pre-addiction period. Urinary and plasma corticoids were decreased during addiction, increased during withdrawal. Administration of ACTH resulted, within 2-3 hr, in a decrease to zero of blood eosinophils and in about the same high level of plasma corticoids during control and addiction periods. The response of urinary corticoids was less, but the increases were similar during control and addiction phases. After infusion of hydrocortisone (2.5 mg/kg body weight), its rate of disappearance from the plasma was not significantly altered during addiction as compared to control periods. In these loading tests the amount of hydrocortisone excreted during 24 hr was comparable during control, addiction, and recovery periods. These results indicate that the catabolism of the hormone is not altered during addiction. Withdrawal was accompanied by a striking rise in plasma and urinary corticoids. The peak plasma corticoids appeared at about 36 hr, the excretion peak on the 2nd day. In several other subjects with more prolonged addiction to higher doses of morphine, much greater and earlier increases in plasma corticoids were noted. Administration of morphine 48 hr after withdrawal relieved symptoms of abstinence within 4 hr and caused a concurrent precipitate fall of plasma corticoids.

762. ISOLATION OF A METABOLIC PRODUCT OF 6-MERCAPTOPURINE FROM HUMAN URINE. Gertrude B. Elion and George H. Hitchings. Wellcome Research Labs. Tuckahoe, N. Y.

The occurrence in human urine of an S^{35}-containing compound of unknown structure after administration of S^{35}-6-mercaptopurine has been reported (Hamilton and Elion. Ann. New York Acad. Sc. 60: 304, 1954). The separation of this product from the other S^{35}-containing metabolites, e.g. sulfate, 6-thiouric acid, and from 6-mercaptopurine itself was accomplished by column chromatography using ion-exchange resins. The compound is acidic enough to be retained on Dowex-1 (formate) and not sufficiently basic to be held on Dowex-50 (H^+). Difficulty was encountered in purifying the unknown from uric acid but this was finally accomplished by repeated paper and column chromatography. The purified product exhibited a ratio of radioactivity to ultraviolet absorption which remained constant after chromatography in a number of solvent systems. The compound is stable to treatment with dilute nitric acid, hydrogen peroxide, 1 N hydrochloric acid and concentrated ammonium hydroxide. However, it is decomposed by 4 N hydrochloric acid after 1 hr in a boiling water bath. Its ultraviolet absorption spectrum exhibits a maximum at 288 mu at pH 1 and at 297 mu at pH 11. In this respect it resembles derivatives of 6-mercaptopurine in which the sulfur bears a substituent. The unknown has been compared with a large number of synthetic compounds closely related to 6-mercaptopurine but, although it exhibits a general resemblance to certain classes of derivatives, it is not identical to any known substance.

763. HIPPURIC ACID EXCRETION IN MAN FOLLOWING SMALL DOSES OF BENZOATE. Howard C. Elliott, Jr.* and Hsien Wu. Dept. of Biochemistry, Med. College of Alabama, Birmingham.

A new micromethod for hippuric acid was used for these studies. It was sensitive to 1 μg and was based on the removal of cationic components of the sample by ion exchange followed by ultraviolet spectrophotometry of hippuric acid in the aqueous effluent. It was found that the rate of excretion of endogenous uric, hippuric and glucuronic acids was constant in a range of 12-18 hr in normal fasting individuals. The data obtained following a small (122 mg) dose of benzoate or hippurate were evaluated in terms of consecutive first order reactions. The constants obtained were based on the assumptions that diffusion equilibria were instantaneously obtained, and that the "glycine pool" was large compared to the benzoate dose. Three constants were evaluated: "K_E" which represents the excretion of hippurate, "K_s" which includes the rate of synthesis of hippurate from benzoate, and "K_a" which includes the absorption of benzoate from the gastrointestinal tract. The average values obtained for the 3 constants following either 1 or 2 mEq doses were as follows: $K_E = 2.69\ hr^{-1}$ or $0.045\ min^{-1}$; $K_s = 9.8\ hr^{-1}$ or $0.16\ min^{-1}$; $K_a = 27.3\ hr^{-1}$ or $0.46\ min^{-1}$. A small number of patients were studied in whom kidney or liver pathology was suspected. The determination "D_E" in cases of kidney pathology indicated that this constant would be affected in some cases. Only "K_s" was affected in patients with liver involvement.

764. QUANTITATIVE STUDIES OF PRODUCTION OF BILE AND BILE ACIDS IN THE RAT. William H. Elliott. Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.

Adult male rats maintained either on ground Purina laboratory chow or on a purified adequate ration were subjected to bile duct cannulation and collection of bile, either immediately after surgery or 48 hr postoperatively. Circulation of bile was maintained in the latter case by external connection of the bile cannula with an inlying duodenostomy tube inserted through the distal portion of the bile duct. Bile volumes were recorded and aliquots taken for cholic acid assay. When bile collection was begun immediately after surgery, the excretory pattern was significantly different from that obtained if enterohepatic circulation of bile was maintained during a 48-hr postoperative recovery period prior to beginning the collection. Under the latter conditions, a maximal level of bile and cholic acid production was

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attained more rapidly. Bile collection from rats on ground chow was continued for as long as 37 days with no observed depression in bile or cholic acid production. The amounts of Acid I and Acid II in pooled bile samples were determined using isotope dilution techniques. Bile from animals on chow diet contained an average of 76 mg of Acid I and 99 mg of Acid II per gram of cholic acid. Acid I or Acid II could not be isolated from the pooled bile obtained from animals on the purified diet.

765. PATHWAYS AND RATE OF HEMOGLOBIN CATABOLISM DURING EXPERIMENTAL HEMOGLOBINEMIA IN SWINE. Arthur J. Emery, Jr., Harold W. Bales, Jr. and Herman E. Pearse (intr. by Robert F. Witter). Flash Burn Section, Univ. of Rochester Atomic Energy Project, Rochester, N. Y.

A surgical technique was developed which permitted the bile to be collected quantitatively or to retain a relatively normal pathway as desired. Urine was also collected quantitatively by means of a catheter. Experimental hemoglobinemia was produced by i.v. injection of various amounts of a porcine hemoglobin solution, simulating hemoglobinemia produced by large surface area, hot water burns. Concentrations of free plasma hemoglobin from 180 to 588 mg % were obtained. Continuous collection of 30-min. samples of bile and urine was carried out over a 12-hr postinjection period. Samples of plasma were obtained every 30 min. The plasma and urine samples were analyzed quantitatively for free hemoglobin and bilirubin and the bile was analyzed quantitatively for bilirubin. The results indicate that free plasma hemoglobin in swine is rapidly catabolized or excreted. Hemoglobin is catabolized and excreted exclusively as bilirubin via the bile when the plasma hemoglobin concentration does not exceed approximately 100 mg of hemoglobin per kilogram of body weight (approximately 200 mg % plasma hemoglobin). The kidney threshold for hemoglobin in swine is approximately 200 mg % and concentrations exceeding this value produce an almost linear increase in hemoglobinuria and bilirubinuria with little or no change in the bilirubin excreted via the bile. The maximum recovery of hemoglobin injected (as hemoglobin and bilirubin) was 76% in the 12-hr period postinjection.

766. INFLUENCE OF CASTRATION AND TESTOSTERONE ON THE GLUTAMIC DEHYDROGENASE ACTIVITY OF MOUSE TISSUES. Gerald L. Endahl (intr. by Max N. Huffman). Oklahoma Med. Research Fndn., Oklahoma City.

Castration of mice at approximately 30 days of age resulted in an increase in the glutamic dehydrogenase activity of the kidney within 30 days and produced a further increase 70 days after castration. The administration of testosterone as a s.c. implanted pellet (15 ± mg) 30 days after castration reversed these changes. The activity of the enzyme in the liver was not altered by either castration or testosterone treatment. The enzyme was detected only in trace amounts in the heart. The preparation of the homogenates in water yielded an approximately 6-fold increase in the liver and 2.5-fold in the kidney over that prepared in 0.05 M potassium phosphate buffer (pH 7.4). The changes due to castration and testosterone treatment, however, were not altered.

767. POTASSIUM FERRICYANIDE AS TERMINAL ELECTRON ACCEPTOR IN OXIDATIVE PHOSPHORYLATION. Ronald W. Estabrook (intr. by A. N. Richards). Johnson Fndn., Univ. of Pennsylvania, Philadelphia.

Chance and Williams (Advances Enzymolo. 17:65, 1956) have described spectroscopic and respiratory responses of mitochondria to changes of ADP concentrations. In the studies reported here $K_3Fe(CN)_6$ is used instead of oxygen as the terminal electron acceptor, and this abbreviated system retains its dependence on ADP concentrations—spectroscopically and catalytically—permitting a simple and convenient assay for an ADP/$K_3Fe(CN)_6$ or (P/e) ratio. For guinea pig liver mitochondria the P/e values are 0.6-1.1 with β hydroxy butyrate, glutamate, malate, or succinate as substrate. A comparison of the affinity for $K_3Fe(CN)_6$ with the influence of concentrations of $K_3Fe(CN)_6$ on the steady state of the various respiratory pigments shows that the principle site of action of $K_3Fe(CN)_6$ is cytochrome c. This has been confirmed in a variety of other ways: The reduction of $K_3Fe(CN)_6$ is inhibited by antimycin A and amytal; the rate of reduction of $K_3Fe(CN)_6$ by succinate, using a preparation devoid of cytochrome c, shows a 3-4 fold increase on addition of cytochrome c; the rate of reduction of $K_3Fe(CN)_6$ under aerobic conditions is not inhibited by CN or azide. The steady states of cytochromes b and c during this phosphorylation process are largely oxidised and this result will be discussed in the light of current hypotheses of the mechanism of oxidative phosphorylation.

768. METABOLISM OF POLYMORPHONUCLEAR LEUCOCYTES. Frances L. Estes, Shirley Smith and Joseph H. Gast (intr. by James B. Walker). Dept. of Biochemistry, Baylor Univ. College of Medicine, Houston, Tex.

By modifications of existing methods, techniques have been developed for obtaining polymorphonuclear leucocytes in good yields from intraperitoneal exudate of guinea pigs. The recovered cells are 85-97% viable at the time of harvest, decreasing to 60-64% after 48 hr. and to 25% after 72 hr. as indicated by vital staining. Incubation of the intact cells in a Warburg apparatus at 37°, pH 7.4 in the presence of glucose indicates: 1) glucose in the absence of other additives suppresses O_2 consumption; 2) the addition of DPN increases O_2 consumption; 3) added cytochrome c alone increases O_2 consumption more than 50%; 4) the addition of both DPN and cytochrome c does not increase the O_2 consumption to the sum of each additive alone and the evidence to date suggests that the frequently observed "aerobic glycolysis" of leucocytes may be due to a defect in the cytochrome system; 5) when iodoacetate is added there are 3 moles of oxygen consumed per mole of pyruvate utilized.

769. EFFECT OF FLUOROCOMPOUNDS ON MITOCHONDRIAL NUCLEOTIDES. Alan S. Fairhurst,* E. M. Gal and R. E. Smith.* Physiology and Pharmacology Depts., Univ. of California Med. Ctr., Los Angeles.

Fluoroacetate, fluoropyruvate and fluorocitrate have been shown to affect oxidative phosphorylation in rat liver mitochondria. This effect can be correlated with the finding that the dephosphorylation of AMP, ADP and ATP by mitochondria is accelerated by the addition of the fluorocompounds. Fluorocitrate appears to have different sites of action than does dinitrophenol, which also stimulates the dephosphorylation of these nucleotides. Addition of dinitrophenol to a fluoroacetate-poisoned mitochondrial system produced an increased inhibition of respiration, not the lowered inhibition reported in kidney mitochondria by Judah and Rees (Biochem. J. 55:664, 1953).

Fluoropyruvate, which has been shown to exist in two allomorphic forms, may exert its effect on the nucleotide dephosphorylating system by combining with -SH groups; however, fluorocitrate has even higher dephosphorylating activity and does not appear to involve an -SH interaction of this type. Examination of the effect of fluoropyruvate and fluorocitrate on the intramitochondrial level of DPN disclosed that fluorocitrate markedly reduced the DPN content, while fluoropyruvate was without effect. These results will be related to the finding that mitochondrial DPN -ase activity, as measured by the destruction of added DPN, is not affected by fluoropyruvate, fluorocitrate or by dinitrophenol.

770. SIALOADENECTOMY AND THE METABOLISM OF CALCIUM IN BONE AND SOFT TISSUES OF MOUSE. Yu-Sheng Louise Feng and Arthur A. Wase (intr. by M. John Boyd). Hahnemann Med. College, Philadelphia, Pa.

Sialoadenectomy has been reported to reduce the total ash, Mg and P of the bones of rats. Extracts from salivary glands, when administered to animals, reduce plasma calcium. Sialoadenectomized mice were prepared and treated as described previously. Radiocalcium was administered i.p. The ca^{45} content of tissues was determined 2 hr after administration. Radioassay was performed with the aid of a gas-flow counter and counting was extended in time to give a 2% statistical error. The ca^{45} incorporation into bone and nearly all other soft tissues was greatest in the sialoadenectomized mice. It suggests that the animals were depleted in calcium. A calcium balance study showed that a reduced intake by the sialex mice, a higher fecal output and a lower urinary output indicate that the absorption of dietary calcium was decreased. The reduced urinary excretion was investigated with ca^{45} and found to be 38% of control values in a 24-hr test. A 2-hr uptake of Sr^{89} showed that the sialex animal's femurs was 175% of control values. It suggests interference with the absorption of calcium from the digestive tract. Other interrelations will be discussed.

771. BIOSYNTHESIS OF β-HYDROXY-β-METHYL GLUTARATE (HMG) IN YEAST EXTRACTS. James J. Ferguson, Jr.* and Harry Rudney. Western Reserve Univ. Med. School, Cleveland, Ohio.

In a study of the reaction in which acetyl CoA (AcCoA) and acetoacetyl CoA (AcAcCoA) enzymatically condense to form HMG (or a CoA derivative thereof), the main obstacle to stoichiometric analysis has been contamination of all enzyme preparations with beta keto thiolase. This contaminant allows interconversion of both substrates by thiolytic cleavage of AcAcCoA in the presence of free CoASH. The latter could be endogenous in the preparations, or supplied by enzymatic deacylation of AcCoA, AcAcCoA, or HMG CoA. Using pressed bakers yeast as a source material, the HMG condensing enzyme has been purified 50-fold, to the point where thiolase activity is greatly reduced. This purification is accomplished by a sequence of toluene lysis of the yeast, adsorption of nucleotide cofactors with Dowex-1 bicarbonate, precipitation of activity with ammonium sulfate, adsorption and elution from calcium phosphate gel, and precipitation in a zinc-alcohol mixture. This purification also provides relative stability for the enzyme. A spectrophotometric assay of the condensation reaction has been devised, utilizing the disappearance of AcAcCoA as measured by its enolate ion absorption at 310 mμ (pH 8.1). Specificity of this assay has been checked at various stages of purification by studying the rate of incorporation of acetyl-1-C^{14} CoA into HMG and reasonable agreement has been obtained. Purified preparations have been found to be free of significant amounts of AcCoA deacylase, and AcAcCoA deacylase. The condensation reaction is specific for CoA esters of acetate and acetoacetate; pantetheine esters were inactive.

772. EFFECT OF STRESS ON TUMORIGENESIS IN RATS. John B. Field, Thomas M. Graham,* Louis T. Bascoy,* William Harris* and Herman M. Harvey.* Depts. of Medicine and Psychology, Univ. of Southern California and Los Angeles County Hosp., Los Angeles.

Over the past decade there has been increasing mention of the effects of stress on cancer growth in humans. However, in the majority of instances studies reported have lacked objectivity either in terms of precise control of the type of stress and/or sampling of variety of tumor types. In the present trials, three types of tumors were used; sarcomas resulting from methylcholanthrene and the transplanted Walker carcinosarcoma and Murphy-Sturm lymphosarcoma. Stresses from 1 to 9 months were applied to Wistar rats. This involved combined inevitable punishment by 0.5-sec electric shock with 12-sec warning signals of a visual nature. Stress was given both before and/or during tumor induction or transplantation. In the 10 different groups of rats studied, results were not suggestive of either a stimulating or inhibiting tumor influence by stress. However, when stress had been applied for 50 days prior to tumor induction or transplantation, there appeared to be an acceleration of tumor incidence. Of 30 stressed rats 15 deaths with tumor growth were noted, while only 8 tumor-induced deaths were seen in a comparable group of 29 unstressed control rats. This suggested a more complex mediating relationship between stress and tumorigenesis, possibly related to the "general adaptation syndrome." In line with this, adrenalectomy or stereotaxic destruction of the median eminence of the hypothalamus was performed. Comparable tumor-stress studies were done on these prepared rats. When these animals were stressed significant alterations in tumorigenesis were noted. For example, adrenalectomized rats subjected to stress yielded a lethal tumor incidence of 1/13 whereas adrenalectomy alone yielded 4/13 and control rats 7/14. (Supported by grants from the Natl. Science Fndn., the Grace McCray and Cora Niles Memorial Funds.)

773. PRELIMINARY METABOLIC STUDIES IN VITAMIN E DEFICIENCY. Kay Fink, Albert D. Williams* and Robert M. Fink. Biophysics and Physiological Chemistry Depts., Univ. of California, Los Angeles, and VA Hosp., Long Beach.

Vitamin E deficiency was produced in weanling female rabbits using the diet of Young and Dinning (J. Biol. Chem. 193:743, 1951). After about 2 wk, the urine showed increasing amounts of 1-methylhistidine (as noted previously in clinical studies by Blahd et al. Proc. Soc. Exper. Biol. & Med. 90:704, 1955), followed by creatinuria and frank muscular dystrophy. Surviving liver from normal and vitamin E deficient rabbits exhibited a similar capacity for hydrolysis of dihydrothymine and dihydrouracil to the corresponding B-amino acids, but the deficient liver was more active in converting thymine-2-C^{14} to 5-hydroxymethyluracil and other radioactive metabolites. Incubation media containing sodium formate-C^{14} and dystrophic muscle showed an abnormal pattern of ultraviolet-absorbing compounds and accumulated several radioactive metabolites, such as methionine,

which were not detectable in control incubations. (Increased incorporation of formate into proteins and nucleic acids of E-deficient rabbits in vivo has been reported by Dinning et al. J. Biol. Chem. 217:205, 1955.) Work in progress involves rechecking tentative identification of some of the metabolites, investigating the metabolism of other tissues, and studying the influence of various additions, including antimetabolites. (Supported in part by Cancer Research funds of the University of California and by PHS Grant C-1669.)

774. SULFHYDRYL GROUPS OF CRYSTALLINE PAPAIN. Bernard J. Finkle* and Emil L. Smith. Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.

Excess p-chloromercuribenzoate (PCMB) reacts reversibly with a maximum of one SH group per mole of papain in dilute aqueous solution, as measured spectrophotometrically. Under optimal conditions for mercaptide formation, papain binds 1 M of Hg^{++}. One equivalent of iodoacetamide irreversibly inactivates papain. These data indicate one reactive SH group in papain and that this group is essential for activity. Preparations of papain isolated from different batches of commercial dried latex possess a variable activity which is directly proportional to the content of PCMB - reactive SH, a maximum of 1.0 SH/M being found in the best preparations (C_1 = 1.8). Inasmuch as less active preparations of enzyme are homogeneous by chemical, physical, immunological and chromatographic criteria, there appear to be two similar forms of crystalline papain, one being nonactivatable and containing a modified, unreactive SH group. Papain, oxidized with performic acid and hydrolyzed, yields 6 residues of cysteic acid. Under certain denaturing conditions, e.g., prolonged exposure to 70% ethanol, as many as 6 M of PCMB react with the protein. These data suggest that disulfide bonds are absent from papain. The significance of these data in terms of the activation and structure of papain will be discussed. Inasmuch as papain contains 8 atoms of sulfur, lacks methionine, and yields only 6 atoms of S as cysteine, there are 2 atoms of S in unknown form. No inorganic sulfate, acid-labile sulfate, or other known amino acids have been detected.

775. PHOSPHOPEPTIDES FROM P^{32}-LABELED PHOSPHORYLASE a. Edmond H. Fischer, Donald J. Graves* and Edwin G. Krebs. Dept. of Biochemistry, Univ. of Washington, Seattle.

In previous reports from this laboratory it was shown that the conversion of muscle phosphorylase b to phosphorylase a by ATP, Mg^{++} and "converting-enzyme" results in a phosphorylation and a dimerization of the protein, with incorporation of 4 gram-atoms of P per mole of phosphorylase a. The fate of the bound phosphate during enzymatic degradation of phosphorylase has been studied. When P^{32}-labeled phosphorylase a is attacked by PR-enzyme, inorganic phosphate is released quantitatively, and phosphorylase b is obtained that can be reconverted to phosphorylase a under the conditions mentioned above. As the whole process can be repeated, catalytic amounts of phosphorylase, converting-enzyme, PR-enzyme and Mg^{++} could enable the complete conversion of ATP to ADP and inorganic phosphate, the system acting as an ATP-ase. When P^{32}-labeled phosphorylase a is attacked by trypsin, the bound phosphate is released in an organic, TCA-soluble form. In this case, the phosphate-free protein produced in the reaction cannot be reconverted to phosphorylase a. Purification of the P^{32}-labeled organic fragment on Dowex 50 followed by paper chromatography in a butanol-acetic solvent has led to the isolation of a pure phosphorylated hexapeptide with the following amino acid composition: Glu, Lys, Arg, Ser, Val, Leu (or Ileu), in a 1 to 1 molar ratio. A second phosphopeptide deriving from the former has also been isolated and studied.

776. STEROID HORMONES AND THE ACETYLATION OF SULFANILAMIDE. A. F. Fishkin and G. F. Lata (intr. by H. B. Bull). Dept. of Biochemistry, State Univ. of Iowa, Iowa City.

Preliminary studies in this laboratory (Franz and Lata. Fed. Proc. 12:204, 1953) have demonstrated that sulfanilamide acetylation by male is significantly greater than by female albino rats; that testosterone propionate enhances female acetylating ability, whereas castration of fully mature males has no significant effect. This sex difference in acetylation is not evidenced by immature male and female rats, immature males having a lower acetylating capacity than mature males. Castration of males at birth lowers, somewhat, the percentage sulfanilamide which can be acetylated by such animals 1-2 months after the operation, whereas in the female ovariectomy of immature animals, has no effect when these animals are tested after 31-42 days. Injection of 200 μg of estradiol-17- /day into mature or immature males will decrease the level of acetylation. This effect evidences itself in mature males in from 5 to 7 days. Injection of 500 μg of testosterone into mature females exerts no significant effect after 30-38 days; increasing the dose to 1000 μg/day for an additional 31-54 days does increase sulfanilamide acetylation. These results have possible significance in the interpretation of androgen-estrogen effects on lipid metabolism.

777. LIVER MALTOSE AND ITS HIGHER HOMOLOGUES: AN UNEXPLORED AREA OF CARBOHYDRATE METABOLISM. William H. Fishman and Hsien-Gieh Sie.* Cancer Research and Cancer Control Unit, Dept. of Surgery and Dept. of Biochemistry and Nutrition, Tufts Univ. School of Medicine, Boston, Mass.

During a study of liver glucosiduronic acids, it was noted quite incidentally that the liver contained an appreciable amount of a mixture of oligosaccharides. It was proven experimentally that these substances are present in this form in liver and are not artefacts of the isolation. They were separated by a chromatographic procedure (charcoal:celite column) and isolated. Upon acid hydrolysis, glucose was found to be the only product. Paper chromatography of the original mixture indicated the presence of 8 components, the three most rapidly moving spots being present in greatest concentration. Employing a combination of analytical procedures, it was established unequivocally that the components of this mixture are maltose, maltotriaose, maltotetraose and higher glycosyl homologues. There is no doubt that this oligosaccharide mixture is metabolically active since it is markedly reduced in the livers of fasted rats and returns to normal levels upon glucose administration. The approximate position of this carbohydrate family in relation to glycogen will be reported. The intriguing problem of the origin of liver maltose is now under investigation.

778. BILIARY EXCRETION AND METABOLISM OF RADIOACTIVE L-THYROXINE. Eunice V. Flock, John H. Grindlay* and Jesse L. Bollman.* Depts. of Biochemistry and Exptl. Surgery, Mayo Fndn.,

Rochester, Minn.

A study has been made of the metabolism of radioactive L-thyroxine in normal dogs and in dogs with either a biliary fistula or biliary obstruction. About 1/3 of the injected I^{131} was excreted in the urine by normal dogs, or dogs with biliary obstruction. An average excretion of 24.2% of the I^{131} in the urine and 21.7% in the bile in 24 hr was found in 8 dogs with biliary fistulas. Fractionation of the iodine compounds on a kieselguhr column showed 6 peaks of radioactivity corresponding to thyroxine, 2 unidentified compounds, iodide, the glucuronide of thyroxine and a water-soluble column residual fraction. Most of the radioactivity in normal urine was present as iodide and not over 5% was found in any one of the organic iodine compounds. Dogs with biliary fistulas or biliary obstruction excreted a smaller percentage of the I^{131} as iodide and more as the conjugate of thyroxine. More of both free and conjugated thyroxine and of the column-residual fraction was excreted in the bile than in the urine. In dogs with biliary obstruction however as much thyroxine conjugate was excreted in the urine as would normally be excreted in the bile. Most of the I^{131} circulating in the plasma 6 hr after the injection of thyroxine was unchanged thyroxine. Even after 24 hr about 2/3 of the I^{131} in the plasma was present as thyroxine and about 1/4 as iodide. The metabolism of thyroxine, like that of triiodothyronine, is not greatly altered by loss or obstruction of bile.

779. GLYCOLYSIS IN THE GROWING AND REGRESSING FLEXNER-JOBLING CARCINOMA. P. J. Fodor, P. Tomashefsky* and C. Funk. Dept. of Biochemistry, New York Med. College, and Funk Fndn. for Med. Research, New York City.

The glycolysis by homogenates from the non-necrotic part of nonregressing (NRT) and of regressing tumors (RT) was studied by CO_2 evolution from phosphate-bicarbonate, lactate production and phosphate uptake and release. The following substrates, cofactors and activators (in micromoles) were contained in a total of 3 ml: glucose 25, HDP 7.5, DPN 0.82, pyruvate 12.6, $MgCl_2$ 16.2 and ATP 0.36. At this concentration ATP is the limiting factor, sustaining the glycolysis of NRT for over 120 min while that of the RT stops after 35-40 min. The average amount of lactate produced in 90 min by NRT is 5.8 μM against 2.7 produced by RT. About 0.5 M of phosphate are released for every mole of lactate produced. ATP can be fully replaced by ADP but when replaced by AMP glycolysis by NRT is decreased by 70%. It has been reported by us (Fed. Proc. 15:253, 1956) that ATPase in RT is increased from 2.5 to 3.5 times. In 0.0083 M of fluoride glycolysis by RT is restored to 80-100%, while in its absence it stops due to the accumulation of 1,3 phosphoglyceric acid. Lactate production in the presence of AMP (or under conditions which favor its accumulation from ATP or ADP) is substantially larger than calculated from the CO_2 evolved, indicating the removal of CO_2 possibly by way of adenosinphosphoryl carbonate (Bachhawat et al. Fed. Proc. 15:214, 1956). Likewise, if ATP is incubated in bicarbonate-phosphate with $MgCl_2$ and homogenate, 2/3 of the CO_2 evolved, due to the splitting of ATP, are reabsorbed again. There is sufficient evidence to indicate that this binding of CO_2 is neither due to a pH effect nor to an uptake of phosphate.

780. A NEW PANCREATIC PEPTIDASE: CARBOXYPEPTIDASE-B. J. E. Folk, Jules A. Gladner and K. Laki (intr. by J. Sendroy, Jr.). Natl. Insts. of Health, Bethesda, Md.

A new enzyme of bovine pancreas, carboxypeptidase-B, has been reported (Folk). In contrast to the extremely slow release of lysine from hippuryl-L-lysine by 3X crystallized classical carboxypeptidase (carboxypeptidase-A) (Hofmann and Bergmann), this new carboxypeptidase rapidly removes lysine and arginine and also ornithine from the C-terminal position of certain synthetic substrates. Furthermore, it has been shown to rapidly release arginine and lysine from the C-terminal position of some protein substrates (Gladner and Laki quoted in Folk). In contrast to the release of lysine and arginine by carboxypeptidase-B, histidine is released from neither protein nor synthetic substrates by this enzyme. Carboxypeptidase-B exists as a zymogen in pancreas and is activated by the action of trypsin at a rate far exceeding the rate of activation of the carboxypeptidase-A zymogen. The hydrolysis of hippuryl-L-arginine by carboxypeptidase-B follows zero order kinetics in contrast to the first order kinetics observed in the carboxypeptidase-A hydrolysis of carbobepzoxy-glycyl-L-phenylalanine. The amino acid residue adjacent to the basic C-terminal residue in the synthetic substrates, hippuryl-L-prolyl-L-lysine and N-benzoyl-β-alanyl-L-lysine, exerts a definite inhibitory effect on the rate of release of the C-terminal basic amino acid by carboxypeptidase-B. The dipeptide, glycyl-L-lysine, is hydrolyzed at a much slower rate by carboxypeptidase-B than is hippuryl-L-lysine whereas hippuryl-D-lysine is resistant to hydrolysis. Steps in the purification of carboxypeptidase-B and its zymogen from acetone-dried powder are discussed.

781. HIGH SPEED CENTRIFUGATION AND DISTRIBUTION OF SERUM CHOLESTEROL. J. C. Forbes, C. O. Watlington,* A. J. Wasserman* and P. D. Camp. Depts. of Biochemistry and Medicine, Med. College of Virginia, Richmond.

The cholesterol which is extracted from lyophilized serum by cold anhydrous chloroform in 3 hr ("readily extractable" cholesterol) has been determined in the serum and subnatant fluid following centrifugation of the serum at 20,000 x g for 2 hr. The difference between the two "readily extractable" cholesterol values is considered to be cholesterol which rose to the surface during centrifugation and probably primarily represents that present in the chylomicrons. A large percentage of the total cholesterol usually rose when the "readily extractable" cholesterol was markedly elevated. In a number of these sera over 50% of the total cholesterol went into the supernatant layer during centrifugation. Rabbits rendered hypercholesterolemic by cholesterol feeding showed a similarly large percentage of the total cholesterol rising to the surface during centrifugation. A striking deviation from this general rule was found in the sera of 5 patients with the nephrotic stage of glomerular nephritis and in several other subjects. Very little of the cholesterol of these sera rose during centrifugation in spite of the fact that the "readily extractable" cholesterol was markedly elevated. The data to be presented indicate that in certain sera a large percentage of the "readily extractable" cholesterol is associated with the chylomicrons, but this is not always the case. Whether other low density fractions, e.g. lipomicrons, account for the remainder of the "readily extractable" cholesterol has not been determined.

782. SEPARATION OF Na AND K TRANSPORT IN KIDNEY SLICES. E. C. Foulkes (intr. by Z. B. Miller). May Inst. for Med. Research, Cincinnati

Jewish Hosp. and Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

These results are part of a study of the chemical nature of ion transport systems. Much information has been obtained in the past with the aid of specific inhibitors. However the movements of Na and K are closely related; their exchange maintains a constant intracellular cation concentration. An inhibitor of such a system may react primarily with either Na or K transport. We have found conditions for studying K accumulation in the absence of simultaneous net Na extrusion. By increasing the Na concentration of Ringer's solution in which Na-enriched, K-deficient slices are suspended, the net Na extrusion is inhibited either by increasing back diffusion or by decreasing extrusion against a concentration gradient. The high Na concentration was obtained by substituting isotonic neutral Na pyrophosphate for NaCl, or by the addition of solid NaCl. Slices in such media continue to respire normally and to accumulate K. In the presence of chloride, K uptake is balanced by Cl uptake. These results confirm the existence of active K transport in kidney slices, independent of active Na transport. Mudge (Am. J. Physiol. 173:511, 1953) has previously demonstrated the independence of K fluxes from Na concentrations. The uptake of K Cl is reduced by protamine to the level predicted from the existence of a Donnan equilibrium across the cell membrane. We have previously reported that protamine inhibits the exchange of K for Na (Fed. Proc. 15:254, 1956). The present results indicate that protamine is a specific inhibitor of K transport. Protamine is not a competitive inhibitor but appears to react irreversibly at some active site involved in K transport.

783. SYNTHESES OF CYTIDINE AND RELATED NUCLEOSIDES. Jack J. Fox, Naishun Yung* and Dina Van Praag.* Sloan-Kettering Inst. for Cancer Research, New York City.

The synthesis of compounds of biological interest has been extended by the application of the mercuri route for the preparation of thymine nucleosides (J. Am. Chem. Soc. 78:2117, 1956) to the synthesis of pyrimidine nucleosides bearing a 4-amino function. Acetylcytosinemercury (I) was condensed with acetobromoglucose to give 1-(tetra-O-acetyl-β-D-glucopyranosyl)-4-acetylaminocytosine (II) in good yield. Deacetylation of II gave 1-β-D-glucopyranosylcytosine identical with that obtained previously by Hilbert-Jansen procedure (J. Am. Chem. Soc. 58:60, 1936). Treatment of I with 2 M of tri-O-benzoyl-D-ribofuranosyl chloride (III) followed by debenzoylation yielded cytidine (IV) which was identical with the natural nucleoside of RNA. Tetra-O-benzoyl-D-xylofuranosyl bromide, when similarly treated with I, afforded 1-β-D-xylofuranosylcytosine (V). An alternate route to IV and V was accomplished via the monochloromercuri derivative of 4-ethoxypyrimidone-2 (VI) which, upon treatment with III, yielded 1-(tri-O-benzoyl-β-D-ribofuranosyl)-4-ethoxypyrimidone-2 (VII). Treatment of VII with alcoholic ammonia gave cytidine in good yields. Since 5-hydroxymethylcytosine occurs in the DNA of T-even bacteriophages preliminary studies have been undertaken toward the synthesis of 5-hydroxymethylcytidine (VIII). Monochloromercuri-5-carbethoxycytosine was condensed with III to give 1-(tri-O-benzoyl-D-ribofuranosyl)-5-carbethoxycytosine which, after debenzoylation with alcoholic ammonia, yielded "ethyl cytidine-5-carboxylate" (IX) and "cytidine-5-carboxamide." These nucleosides offer many possibilities, one of which is the reduction of IX to VIII and structural analogs thereof. Investigations on the synthesis of 5-nitro and 5-amino analogs of cytidine from 5-nitrocytosine will be discussed.

784. AN UNUSUAL HIGH MOLECULAR WEIGHT PROTEIN COMPONENT IN THE SERUM OF CERTAIN PATIENTS WITH RHEUMATOID ARTHRITIS. E. C. Franklin,* H. R. Holman,* H. J. Muller-Eberhard* and H. G. Kunkel. Rockefeller Inst. for Med. Research, New York City.

Normal human serum contains a protein fraction with a sedimentation constant of approximately 19S. Materials with an s rate greater than this are not detectable in significant amounts in fresh normal serum. However, observations on sera of individuals with rheumatoid arthritis indicated the presence of a more rapidly sedimenting component in 12 out of 30 cases. In 6 of these it could be quantitated and varied in concentration from 75 to 340 mg%. The calculated s rate of this component was 21-23S. 38 control sera including samples from diseases associated with hyperglobulinemia failed to reveal a similar component. Evidence was obtained that this fraction was involved in certain of the agglutination activating reactions of rheumatoid arthritis sera. In general, the largest amount was contained in sera that were most positive by the sensitized sheep cell agglutination test and the γ globulin precipitation test, while the component was not visible in weakly positive sera. In the γ globulin precipitation test only altered γ globulin, usually obtained by heating at 63°C for 10 min, resulted in a precipitate. Repeated absorptions with altered γ globulin removed the component as well as the biological activity. The high molecular weight component could be purified by preparative ultracentrifugation. It migrated as a γ globulin and readily dissociated in 4-6 molar urea to 2 subfractions with s rates of approximately 19S and 7S. The relationship of this protein complex to other 19S γ globulins and 19S antibodies is under investigation.

785. COMPARISON OF ACUTE EFFECTS OF INSULIN AND AN ARYLSULFONYLUREA ON THE DISPOSITION OF GLUCOSE AND ON BLOOD PYRUVATE. Thomas F. Frawley (intr. by Marjorie R. Stetten). Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

The dosage of intravenous sodium tolbutamide (orinase), an arylsulfonylurea, required to produce in the same normal fasting subjects an hypoglycemia equivalent to that of intravenous insulin was determined. Eighteen milligrams to 40 mg/kg is injected in 3 min and like insulin (0.1 U/kg) produces a 50% or greater decrease in blood sugar in 30 min. Blood pyruvate during this period decreases after tolbutamide but increases characteristically after insulin. Plasma phosphate decreases after both. An oral glucose load (0.8 gm/kg) at the point of maximum hypoglycemia (insulin-glucose tolerance test versus tolbutamide-glucose tolerance test) was also tested. Normally glucose magnifies the homeostatic response to insulin hypoglycemia. With tolbutamide this hyperglycemic response was suppressed and also the blood pyruvate response. Simultaneous injections of glucose (0.3 gm/kg) with tolbutamide or insulin were also compared. The glucose disappearance rate (log of blood glucose concentration versus time) is a straight line with slope K which averages normally 1.8 %/min. In 1 of the subjects K was 7.2 %/min after insulin and 4.0 %/min after tolbutamide. The different pyruvate responses to insulin and tolbutamide administration suggest the acute hypoglycemic effect of tolbutamide is not dependent upon the release of or enhancement of insulin. The glucose disappearance rates and phosphate changes after tolbutamide as generally interpreted suggest increased insulin activity. The hypoglycemic effects of the arylsulfonylureas involve more than a single mechanism of action.

786. INHIBITION OF COPPER ION AND COPPER ENZYME CATALYSES BY CUPROUS ION REAGENTS. Earl Frieden, George Ezell* and Yashvant Karkhanis.* Dept. of Chemistry, Florida State Univ., Tallahassee.

Explorations are in progress on the mechanism of the inhibition of several copper enzyme and copper ion catalyses by specific cuprous ion reagents, such as 2,9-dimethyl-1,10-phenanthroline. The copper ion catalyses studied include the copper ion catalysis of ascorbic acid and the copper enzymes, polyphenol-oxidase and ascorbic acid oxidase. The results are consistent with the inhibition of a cuprous ion or a cuprous-protein intermediate. The inhibition of both the cupric ion catalyzed oxidation of ascorbate and of polyphenoloxidase by the specific cuprous reagent, 2,9-dimethyl-1,10-phenanthroline, appears to be "uncompetitive." A 1/v versus 1/s plot with and without cuprous reagent yields lines of different intercepts but comparable slopes. If the true substrate for these catalysis is regarded as an electron, then the cuprous reagent is reacting exclusively with the enzyme-substrate complex. Therefore, a reaction paralyzing the catalytic species containing cuprous ion should lead to kinetics fitting "uncompetitive inhibition." The reactions may be represented as:

$$Cu^{++}(\text{enzyme}) + AH_2 \longrightarrow Cu^{+}(\text{enzyme}) + AH\cdot + H^{+}$$

$$\text{cuprous reagent} \downarrow$$

$$\text{(inactive) } Cu^{+} \text{ complex}$$

Significant differences were also found between the inhibiting effects of cupric ion reagents and cuprous ion reagents on the cupric ion catalyzed oxidation of ascorbic acid. (Aided by Natl. Science Fndn. Grant G-1037.)

787. FRACTIONATION OF RELAXIN PREPARATIONS BY COUNTER-CURRENT DISTRIBUTION. Edward H. Frieden and Noel W. Layman.* Arthritis Research Lab. and Cancer Research and Cancer Control Unit, Tufts Univ. Med. School, Boston, Mass.

Relaxin-containing extracts of ovaries of pregnant sows, when distributed in the system: 1% trichloro-acetic acid/s-butanol, display 4 major components, with distribution coefficients of 0.03, (a), 0.45 (b), 1.8 (c) and 9 (d), respectively. The distribution of relaxin activity agrees closely with that expected for a single molecular species; the location of the relaxin peak, however, corresponds to a K_{di} of 2.7, and does not coincide with any of the major components. Relaxin preparations which have been purified by treatment with oxycellulose are practically devlid of components b and c, whereas preparations which have been chromatographed on Dowex-50 consist principally of component d. In the system: 0.1% TCA/s-butanol the position of the relaxin peak has been found to vary between 0.2 and 0.8, depending upon concentration and pH. The behavior of purified preparations in this system suggests that association between some of the components of the mixture may be responsible for part of the apparent inhomogeneity observed.

788. ENZYMATIC CONVERSION OF DEOXYURIDYLIC ACID TO THYMIDYLIC ACID AND THE PARTICIPATION OF TETRAHYDROFOLIC ACID. Morris Friedkin. Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, Mo.

Previous experiments (J. Biol. Chem. 220:653, 1956) showed that deoxyuridine is a precursor of thymidine of DNA through reactions inhibited by Aminopterin and augmented by 5-formyl-tetrahydrofolic acid. On the assumption that the primary acceptor of the methyl group is a phosphorylated form of deoxyuridine, P^{32} - labeled deoxyuridine-5'-phosphate (DU5P) was prepared. Phosphorylation of DU5P to deoxyuridine triphosphate could not be catalyzed by the E. coli kinase (Kornberg et al. Fed. Proc. 15:291, 1956) which rapidly converts thymidine-5'-phosphate (T5P) to thymidine triphosphate (TTP). Only after methylation of DU5P to T5P could the specific T5P kinase catalyze phosphorylation to TTP. This specificity, which perhaps explains why thymine and not uracil is a component of DNA, was made the basis of an assay for the enzymatic conversion of DU5P to TTP. A sample is treated with phospho-monoesterase which dephosphorylates DU5P or T5P but not TTP. TTP if present is adsorbed on charcoal and its P^{32} counted. Charcoal adsorbs nucleotides but not inorganic orthophosphate. Cell-free extracts of E. coli, treated for the removal of cofactors by passage over a Dowex-1 formate column, converted P^{32} - labeled DU5P to TTP in a system requiring tetrahydrofolic acid (THFA), ATP, Mg^{++} and either serine or formaldehyde as 1-carbon donors. Hydroxymethyl-THFA (Kisliuk. Fed. Proc. 15:289, 1956) could substitute for THFA and 1-carbon donors. Thymidine-C^{14} was isolated from similar incubation mixtures containing C^{14} - labeled DU5P. (Aided by Natl. Cancer Inst., PHS grant C-2166.)

789. CARBAMYLATION OF PUTRESCINE. Samuel Friedman (intr. by H. E. Umbarger). Biological Research Labs., Harvard School of Dental Medicine, Boston, Mass.

In the presence of putrescine (P), carbamyl phosphate (CAP) and adenosine diphosphate (ADP), extracts of either bakers' yeast or the putrescine-requiring auxotroph Aspergillus nidulans pu_1 (Sneath. Nature 175:818, 1955) catalyze the following reactions:

$$P + CAP \longrightarrow PCA + Pi \quad (a)$$

$$Pi + ADP \xrightleftharpoons{Mg^{++}} ATP \quad (b)$$

Reaction (a) results in a mixture of the mono- and dicarbamylated amines. The carbamylation of putrescine appears specific since no detectable reaction was observed with CAP and the following amines: 1,3-diamino-2-propanol, cadaverine, histamine, benzylamine, isoamylamine, isobutylamine, glucosamine, ethanolamine, hydroxylamine. Extracts of the putrescine-requiring auxotroph also catalyze the condensation of CAP with ornithine to form citrulline indicating that this amino acid is a probable intermediate in arginine biosynthesis in this species and that the reported inability of ornithinine-arginine requiring auxotrophs to utilize citrulline (Pontecorvo et al. Advances Genet 5:141, 1953) are due to its impermeability. The possible role of the above reaction in the biosynthesis of agmatine and arcaine, and as a detoxification mechanism is under investigation.

790. ANTIMETABOLITES AND PRODUCTION OF MENTAL ABNORMALITIES. Charles E. Frohman, Jacob L. Chason,* Joan Havelena* and Jacques Gottlieb.* Lafayette Clinic and Wayne State Univ., Detroit, Mich.

The following experiments were performed to determine the effects of various antimetabolites, administered to pregnant rats, on the mental development of their offspring. The antimetabolites used

were oxythiamine, isonicotinic hydrazide, indole 3-acetic acid, tryptamine and methionine sulfoxide. A group of at least 10 pregnant rats was fed each antagonist. A 6th group of 10 pregnant animals placed on a stock diet was used as a control. Immediately following their impregnation the rats were placed on diets containing antimetabolites. The antimetabolite was then continued until the 7th day of pregnancy. At that time the animals were returned to a stock diet. On the 7th day 1 animal from each group was killed to determine if the antagonist was passing through the placental barrier. On the 7th day the learning ability and emotionality of the rats were also tested. At the time of delivery, gross abnormalities in the offspring were noted; 1 animal from each group was killed and the brain studied histologically. A 2nd animal was frozen in liquid air, its brain removed and the following materials determined chemically: pyruvic acid, lactic acid, α ketoglutaric acid, glutamic acid, serotonin, 7-min hydrolyzable phosphate, indole 3-acetic acid and tryptophane. Every week thereafter, animals from each group were studied histologically and chemically until the supply of animals was exhausted. At the end of the 3rd and 6th wk, the rats' behavior was assessed by a psychologist. Results will be discussed.

791. INCORPORATION OF N-15 INTO FIBRINOGEN DURING INDUCED NITROGEN STORAGE OR LOSS. O. H. Gaebler, Rachel Glovinsky* and Trieste Vitti.* Edsel B. Ford Inst. for Med. Research, Detroit, Mich.

In a normal bitch receiving a constant diet, incorporation of N-15 into fibrinogen was studied during: 1) nitrogen equilibrium in the untreated animal, 2) nitrogen storage induced with growth hormone and 3) nitrogen loss induced with corticotropin. Atoms % excess of N-15 in fibrinogen nitrogen, determined 3, 6, 12, 24 and 48 hr after ingestion of uniform amounts of labeled glycine was virtually identical under the 3 widely different conditions, in which percentage of ingested N-15 excreted during 48 hr was 46.3, 21.6 and 51.7, respectively. The amount of N-15 present in fibrinogen per 100 cc of blood increased during induced nitrogen storage and decreased during induced nitrogen loss. In an experiment in which N-15 labeled alanine was used in the same untreated animal, 50.5% of ingested N-15 was excreted during 48 hr and the maximum net incorporation into fibrinogen was unexpectedly low, 0.015 atoms % excess, as against .046 in the experiments with equivalent amounts of labeled glycine. Effects of growth hormone on metabolism of N-15 labeled alanine are being studied, in view of the effects of this hormone on hepatic glutamic pyruvic transaminase activity.

792. TRANSAMINASE INHIBITION BY HYDROXY-ASPARTIC, DIAMINOSUCCINIC AND DIAMINO-PROPIONIC ACIDS. Mario Garcia-Hernandez* and Ernest Kun. Dept. of Biochemistry and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

It was reported that oxaloglycolic and diketosuccinic acids were among the products of the enzymatic oxidation of tartrate. An interesting possibility was suggested, that transamination of these keto acids result in amino acids which could serve as physiological regulators of cell metabolism (J. Biol. Chem. 218: 201, 1956). So far, it has been found, by using extracts from acetone powder of pig kidney mitochondria, that glutamate reacts with oxaloglycolate to yield hydroxyaspartate and α-ketoglutarate. Sallach (Fed. Proc. 15:344, 1956) has observed the same reaction. The reverse reaction is faster and therefore the time sequence of transamination was measured by quantitative determination of glutamic acid which was formed from hydroxyaspartate and α-ketoglutarate. Dihydroxyfumarate formation from hydroxyaspartate can be followed spectrophotometrically at 290 mμ. The enzyme preparation used in these experiments is very rich in aspartic-α-keto-glutaric transaminase. It has been assumed that if the same enzyme performs the transamination of both aspartic and hydroxyaspartic acids, and if the 2nd reaction is very much slower, then hydroxyaspartate should act as an inhibitor. Kinetic studies have shown that hydroxyaspartate is a competitive inhibitor. On the other hand diamino-succinate and diamino-propionate showed a different type of inhibition. Hydroxyaspartate and diaminosuccinate in equimolar concentrations with aspartate inhibit transamination by 50%, while diaminopropionate is slightly less effective.

793. POSSIBLE URINARY METABOLITES OF ESTROGENS. Josephine B. Garst. Dept. of Physiological Chemistry, School of Medicine, Univ. of California, Los Angeles.

Previous work done by this author has suggested that the true urinary estrogen metabolites are hitherto unrecognized compounds. That these compounds are not artifacts of known estrogens produced by the method of extraction was indicated by early work which showed that crystalline estrogens could be recovered unchanged from aqueous solution. Present work on the recovery of estradiol added to urine proves this contention. Comparison was made of the properties of the chief component of the possible estrogen metabolites with those of a synthetic oxidation product of estradiol. This synthetic product was formed by the action of oxygen on estradiol in aqueous solution of pH 8-9 for 2 wk. The 2 substances have similar color and solubility. The ultraviolet absorption curve of the synthetic product is like that of an early stage of the progressive change previously demonstrated in this laboratory for the curve of estrone when exposed to air in the presence of riboflavin and light. The curve for the urinary component is similar to that of a later stage of this same change. A study made of the effect of age and of pregnancy on the 24-hr excretion of these possible estrogen metabolites by human beings suggests that a relationship exists between these compounds and the estrogens. In order to test the validity of this suggestion, the effect of exogenous estradiol on the excretion of these compounds by a normal, young woman is being determined. Estradiol labeled with tritium in ring A is being prepared for use in a similar study.

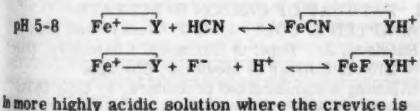
794. A PASTEUR EFFECT IN A RECONSTRUCTED SYSTEM. S. Gatt (intr. by S. Ratner). Public Health Research Inst., New York City.

A reconstructed system, composed of the purified enzymes and coenzymes of glycolysis, combined with respiring mitochondria (Fed. Proc. 15:259, 1956), has been used in the study of inhibition of respiration by glycolysis (the "Crabtree Effect"). The inhibition of glycolysis by respiration (the "Pasteur Effect") has now been studied using the same system. At 3×10^{-4} M ATP, 2.5×10^{-2} M inorganic phosphate (P_i) and 5 mg mitochondrial protein/ml, addition of the glycolytic system resulted in a marked inhibition of respiration, while lactate formation was only slightly inhibited by the presence of the mitochondria. At a mitochondrial concentration of 10 mg protein/ml, there was no

inhibition of respiration, while an 80% inhibition of lactic acid formation was observed. There was however little or no suppression of hexose uptake; the difference in the lactic acid levels could be accounted for by increased accumulation of fructose-1,6-diphosphate. The effect of decreasing the P_i concentration and adding ATPase was then studied. At low phosphate (6×10^{-4} M) a maximal rate of glycolysis was obtained within a rather narrow range of ATPase concentrations. At these ATPase concentrations, addition of mitochondria resulted in a 50% inhibition of glucose uptake and 80% inhibition of lactate formation. This was accompanied by a marked drop in the final concentration of P_i. The above findings suggest that competition between mitochondria and glycolysis for limiting amounts of ADP results in a decrease of lactate formation. When the 2 systems compete for both adenine nucleotides and inorganic phosphate, a pronounced inhibition of hexose uptake can also be obtained.

795. POSSIBILITY THAT THE HEME IS IN A CREVICE IN FERRIPEROXIDASE AND FERRICATALASE. Philip George and R. L. J. Lyster (intr. by D. R. Goddard). John Harrison Dept. of Chemistry, Univ. of Pennsylvania, Philadelphia.

The pH variations of the equilibrium constants for hemoprotein complex formation fall into 2 distinct classes. For example, with the fluoride complexes the constants for ferrimyoglobin and ferrihemoglobin are approximately independent of $[H^+]$ in the pH ranges 5-8 and 5-7, whereas those for ferriperoxidase and ferricatalase are approximately directly proportional to $[H^+]$: with the cyanide complexes over the same pH ranges, the constants for ferrimyoglobin and ferrihemoglobin are approximately inversely proportional to $[H^+]$, whereas those for ferriperoxidase and ferricatalase are independent of $[H^+]$. This behavior indicates a fundamental difference in the composition and structure of either the parent hemoproteins or their complexes, whereby, if anions are bonded in the complexes, a proton is consumed for each molecule of complex formed with peroxidase and catalase, but not with myoglobin and hemoglobin. Previously, explanations have been sought in terms of structures where the 6th coordination position of the iron atom is occupied by OH^- instead of H_2O in ferriperoxidase and ferricatalase, or by the acids HF, HCN, etc. instead of the anions F^-, CN^-, etc. in their complexes. The participation of heme-linked ionizations has also been considered. Another possibility is that the heme is situated in a crevice in ferriperoxidase and ferricatalase with the weaker bond to a group having a high proton affinity, so that when complex formation occurs in a pH range on the acidic side of its pK a proton is consumed:



in more highly acidic solution where the crevice is broken giving $Fe^+(H_2O)Y$ H^+, and in solutions alkaline to the pK of YH^+, the pH behavior should revert to that chatacteristic of ferrimyoglobin and ferrihemoglobin. Experimental data are reviewed to test this hypothesis.

796. MOLECULAR PARAMETERS OF CARDIAC MYOSIN. J. Gergely and H. Kohler.* Cardiovascular Research Lab., Dept. of Medicine, Massachusetts General Hosp., and Harvard Med. School, Boston.

Crystalline, actin-free, myosin was prepared from dog hearts, excised, while still beating, under anesthesia, by extraction with KCl-pyrophosphate. Actin was removed by precipitation at $\Gamma/2 = 0.3$ followed by ultracentrifugation. The myosin was precipitated 4 times at $\Gamma/2 = 0.06$. The light scattering of the myosin solutions was measured at angles ranging from 30 to 135° in a cell, recently described by Dandliker and Kraut (J. Am. Chem. Soc. 78:2380, 1956), permitting centrifugation at 55,000 x g. The measurements were carried out at several concentrations, and the results were evaluated according to Zimm, yielding a molecular weight of $5.0 \times 10^5 \pm 10\%$, and a root-mean-square radius of 470-500 A. The length of the molecule, determined from measurements of double refraction of flow, is 1600-1700 A. The combination of these results indicates a rod-like structure. Taking into account the fact that the axial ration calculated from the intrinsic viscosity is 65-68, one arrives at a width of 23-24 A. These values are in good agreement with our own measurements on skeletal myosin, and with those recently reported for the skeletal protein by Holtzer and Lowey (J. Am. Chem. Soc. 78:5955, 1956). Our results appear to be at variance with those of Stern et al. (Fed. Proc. 15:764, 1956), who reported molecular weights for cardiac myosin of the order of 2×10^5. A comparison of ATPase activities - Q_p = 1000-1200 for our preparation, 150 for that of Stern et al. - would however suggest that the apparently lower molecular weight might conceivably be due to impurities in that preparation.

797. A COMPARISON BETWEEN PORCINE AND BOVINE MELANOCYTE-STIMULATING HORMONES. Irving I. Geschwind,* Choh Hao Li and Livio Barnafi.* Hormone Research Lab., Univ. of California, Berkeley.

The isolation and structure of a melanocyte-stimulating hormone (β-MSH) from posterior-intermediate lobes of porcine pituitary glands has previously been reported. By means of an isolation procedure similar to that employed for the isolation of β-MSH, a related bovine MSH has been obtained. In the course of the isolation, it was observed that the mobility of the bovine preparation in starch electrophoresis is different from that of the porcine hormone. Furthermore, in countercurrent distribution (system: 2-butanol vs. aqueous 0.5% trichloroacetic acid) the partition coefficient of the bovine hormone was smaller than that of β-MSH. Amino acid analysis has shown that the bovine MSH possesses 1 more residue of serine and 1 less residue of glutamic acid than does the porcine MSH. This replacement of an acidic residue by a neutral one is reflected in the more basic isoelectric point of the bovine MSH (7.0 vs. 5.8). Chymotryptic hydrolysis of the bovine MSH produced 4 peptides; by means of the general degradation techniques now available, the structure of these peptides and consequently of the hormone, was elucidated to give the following sequence for the bovine MSH: Asp. Ser.Gly.Pro.Tyr.Lys.Met.Glu.His.Phe.Arg.Try.Gly. Ser.Pro.Pro.Lys.Asp. This sequence will be compared with that of the porcine hormone. In addition, the effect of alkali-heat treatment upon the activities of the 2 hormones will be reported. (Supported in part by research grants from the Natl. Insts. of Health, PHS G-2907.)

798. INFLUENCE OF GLUCOSE ON THE DISTRIBUTION OF C^{14} IN SUGAR FORMED DURING PHOTOSYNTHESIS. Martin Gibbs and Otto Kandler.* Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y., and Botanical Inst.,

Univ. of Munich, Munich, Germany.

A symmetrical distribution of C^{14} in the hexoses formed during photosynthesis has been reported. However, Kandler and Gibbs (Plant Physiol. 31:411, 1956) observed in Chlorella after photosynthesis in $C^{14}O_2$ an unsymmetrical distribution of tracer in the glucose moieties of the phosphate esters. In the present investigation, photosynthesis in $C^{14}O_2$ was carried out by Chlorella at the compensation point in the absence and presence of a supply of unlabeled glucose. Samples of glucose isolated from sucrose and starch after periods of 15, 45 and 180 min were degraded with Leuconostoc mesenteroides. In the absence of exogenous glucose, essentially symmetrical distribution in the glucose moieties was observed but the presence of the C^{12}-glucose resulted in an unsymmetrical distribution (C-1, 17%, C-2, 13, C-3, 25, C-4, 31, C-5, 7, C-6, 8) after 180 min. When glucose-1-C^{14}, glucose-2-C^{14} or glucose-6-C^{14} were fed to Chlorella in the dark or at the compensation point for 80 min and the glucose moiety of the sucrose was degraded, the tracer distribution was similar for each sugar under both conditions: about 70 - 80% was retained in the site of the original label with approximately 25% becoming symmetrically located in the other triose moiety of the glucose. These data suggest that although exogenous glucose can enter the photosynthetic cycle, a mechanism is present which returns most of the carbon of the exogenous sugar to its original site. (Supported by a grant from the Natl. Science Fndn.)

799. INCORPORATION OF GLYCEROL-1,3-C^{14} INTO TRIGLYCERIDES AND PHOSPHATIDES OF HUMAN SERUM AND SERUM LIPOPROTEINS. Lewis I. Gidez* and Howard A. Eder. Med. Dept., Brookhaven Natl. Lab., Upton, N. Y., and Dept. of Medicine, State Univ. of New York, College of Medicine at New York City, Brooklyn.

Previous studies in rats have shown that C^{14}-glycerol is incorporated into blood and tissue lipides. In the present investigation C^{14}-glycerol has been utilized to study the metabolism of triglycerides and phosphatides of serum and serum lipoproteins in man. One hundred ninety microcuries of glycerol-1,3-C^{14} (77 mg) were given by mouth to a patient with idiopathic hyperlipemia. Frequent blood samples were obtained, and the serum was separated by ultracentrifugal flotation into fractions of densities less than 1.006, 1.006 - 1.063, and greater than 1.063. The lipides were extracted from the serum and lipoprotein fractions and phosphatides were separated from triglycerides. The lipides were hydrolyzed and the formaldehyde resulting from periodate oxidation of the glycerol or glycerophosphate moieties was precipitated with dimedon. The recrystallized formaldimedon was counted in a single phototube liquid scintillation counter. The activity of serum triglycerides was maximal at 4 hr, and its decline was rapid with half-maximal activity occurring at 16 hr. The peak activity of serum phosphatides was 1/6 that of the triglycerides and was not reached until 10 hr. Half-maximal activity was at 60 hr. At 7 hr the specific activity of the beta-lipoprotein triglycerides was about 2.4 times that of the alpha-lipoprotein triglycerides and it declined at a greater rate than did that of the latter fraction. (Supported by the Atomic Energy Comm.)

800. BIOSYNTHESIS OF DIAMINOPIMELIC ACID. Charles Gilvarg. Dept. of Biochemistry, New York Univ. College of Medicine, New York City.

Through the use of the penicillin technique a new diaminopimelic acid requiring mutant of E. coli was isolated. This organism accumulates a compound in its medium that was isolated as an alcohol insoluble barium salt. The accumulated compound was characterized as N-succinyl-L-diaminopimelic acid. Extracts prepared from the parental wild type strain were found to contain an enzyme which catalyzed the deacylation of N-succinyl-L-diaminopimelic acid. This enzyme could not be detected in extracts prepared from the mutant which accumulates N-succinyl-L-diaminopimelic acid. These findings provide evidence that N-succinyl-L-diaminopimelic acid is an intermediate in the biosynthesis of diaminopimelic acid. In a study of the deacylase Co^{++} was found to activate resolved preparations of the enzyme to the greatest extent, though Mn^{++} and Zn^{++} were also found to be rather effective. With the availability of all the isomers of β-hydroxydiaminopimelic acid (obtained from J. Stewart and D. W. Wooley) it has been found that a mutant blocked early in the biosynthesis of diaminopimelic acid will respond to 3 of the 4 racemic pairs. However, only the requirement for diaminopimelic acid can be met by these compounds; they will not meet the lysine requirement. This suggests that they are effective as analogues of diaminopimelic acid rather than by conversion to diaminopimelic acid.

801. LATENT PERIOD OF PANCREATIC ESTERASE ACTIVITY. E. C. Gjessing. Univ. of Vermont College of Medicine, Burlington.

Assaying for esterase (or lipase?) in the pancreas using as substrates aliphatic esters of salicylic acid, (Hafstee. Science 114:128, 1951) it was found that the activity is zero or very close to zero in homogenates, or extracts of the tissue removed immediately after death. After storage of the tissue as well as its homogenates and extracts at 0°-5° for a period of 1 to 3 days, high activity values are attained. The rate of activity increase in homogenates appears to be independent of method of preparation. Homogenates prepared with sucrose in a Potter-Elvehjem type homogenizer, as well as homogenates prepared with water in the Waring Blendor, have no or very low initial activity but upon storage attained high activities and at the same rate. Particulate free extracts obtained from such freshly prepared homogenates by high-speed centrifugation showed the same activation with time as the homogenates. Homogenates prepared from fresh tissue frozen at -20°C, for weeks showed the same rate of activation as the unfrozen homogenates. Work presently in progress to ascertain if the activation is related to lysis of some particulate matter, which the present data does not indicate, or if it is due to an enzymatic activation, will be discussed.

802. PATHWAY OF CARBON IN ACETATE-ADAPTED E. COLI. Alvin J. Glasky* and Max E. Rafelson, Jr. Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago.

Utilizing a standardized procedure, E. Coli (ATC 8739) was adapted for growth on a medium containing inorganic salts and sodium acetate. The growth of the adapted bacteria was excellent on the forementioned medium, yielding 2.5 gm of cells (wet wt.) per liter. Radioactive acetate was administered to the adapted cells during the log phase of their growth cycle. Such cultures were allowed to metabolize the radioactive substrate for varying periods of time and acetone-dilute acid extracts were prepared from the bacteria. Radiochromatographic techniques were employed to follow the progression with time of radioactivity through various cellular metabolites. In time periods

of less than 1 min, succinic and glutamic acids contained the greatest percentage of the radioactivity in the extract. In subsequent time periods, the percentage of the radioactivity in succinate decreased and other compounds (alanine, aspartate, glycine, hydroxypuruvate, tartrate and several unidentified compounds) became appreciably radioactive. In the early time periods, little or no radioactivity was detected in the following compounds: citrate, fumarate, isocitrate, malate and serine. The significance and possible implications of these results will be discussed.

803. PYRUVIC OXIDASE OF MYCOBACTERIUM TUBERCULOSIS. Dexter S. Goldman. VA Hosp., Madison, Wis.

A soluble pyruvic oxidase has been obtained from the H37Ra strain of Mycobacterium tuberculosis var. hominis. The enzyme, solublized during the sonic disruption of the bacterial cells, has been shown to catalyze the oxidation of puruvate to acetate using, equally well, oxygen or ferricyanide as electron acceptor. Cocarboxylase and Mg^{++} are required for this oxidation. The enzyme also catalyzes the oxidative decarboxylation of pyruvate, in the presence of CoA, DPN and cocarboxylase, to acetyl CoA. Acetoin formation has not been detected. The pyruvic oxidase is inhibited by versene but not by p-chloro-mercuribenzoate or arsenite. Further details concerning this enzyme and the oxidative metabolism of the mycobacteria will be discussed.

804. DISTRIBUTION OF THE PROTEOLYTIC ENZYME SYSTEM OF SKIN. Julius Golubow (intr. by W. S. McEllroy). Biochemistry Dept., Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

Studies in this laboratory have revealed the presence of several proteolytic enzymes in rat skin (Martin. Fed. Proc. 15:455, 1956; Martin and Axelrod. J. Biol. Chem. in press). Proteinase A hydrolyzes casein and acetyl-L-tyrosine ethyl ester (ATEE), both activities being suppressed by an inhibitor isolated from blood. A synthetic substrate for proteinase C has not been found, but its presence can be demonstrated, with casein as the substrate, by the use of a reversible inactivation induced by ionic strength changes. Enzymes A_1 and A_2 are amino acid esterases, hydrolyzing, respectively, ATEE and L-tyrosine ethyl ester (TEE); neither of these enzymes can be inhibited by the blood inhibitor. Since both A and A_2 attack TEE, it has been possible to calculate the A_2 concentration of a skin extract from the knowledge of the ratio of the activities of proteinase A toward ATEE and TEE. From the above facts a differential assay procedure was developed for the determination of the proteolytic enzyme system of rat abdominal skin. A survey of the enzyme content of the abdominal skins of various other animals has been performed to select an experimental animal in which skin enzyme distributions could be studied as the result of physiological changes such as nutritional deficiencies, hyperthermia and hypersensitivity. These studies could also be used to find an animal skin more suitable to large scale preparative procedures. Data will be presented showing the distribution of these proteolytic enzymes in normal rat, guinea pig, rabbit, monkey, human, cow, sheep, hog, dog and chicken skins.

805. METABOLISM OF PURINE. Milton Paul Gordon,* Orsalia Intrieri* and George Bosworth Brown. Sloan-Kettering Div. of Cornell Univ. Med. College, New York City.

Purine-8-C^{14} (2.23×10^5 c.p.m./μM) was administered intraperitoneally to 2 rats at the level of 4.43 μM/animal. The animals were bearing 2-wk-old implants of Flexner-Jobling carcinoma. After 24 hr, small, but significant, amounts of radioactivity were found in the adenine and guanine of the pentose and deoxypentosenucleic acids of the liver, intestine and kidney. Only trace amounts of radioactivity could be detected in the adenine and guanine of the nucleic acids of the tumor. There was no evidence that unchanged purine was incorporated into the nucleic acids of any tissue examined. The injected material was extensively catabolized and only 0.7% of the radioactivity injected was found in the perchloric acid soluble material from the viscera. In a similar experiment when the same molar dosage of 9-β-D-ribofuranosyl-purine-8-C^{14} was administered, 17% of the injected radioactivity was found in the acid soluble fraction from the viscera. In 2 such 24-hr experiments the allantoin contained only 50-65% of the radioactivity found in the urine. When high doses of purine-8-C^{14} (2 mM/kg) were administered and the animals killed after 1 hr, radioactive hypoxanthine, xanthine, uric acid and 2,8-dioxypurine were extractable from the kidney.

806. SEQUENTIAL BLOCKADE IN ADENINE BIOSYNTHESIS BY A SINGLE MUTATIONAL EVENT IN ESCHERICHIA COLI. Joseph S. Gots and Edith G. Gollub (intr. by M. G. Sevag). Dept. of Microbiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.

An adenine requiring mutant of Escherichia coli (strain B-97) has been found which is apparently impaired in 2 separate reactions along the biosynthetic pathway to nucleic acid adenine. Its specific growth requirement for adenine (or adenosine or adenylic acid) indicates an inability to convert inosinic acid to adenylic acid. Under growing conditions, or with nonproliferating cell suspensions, an aminoimidazole ribotide is accumulated in high concentrations from glucose and ammonium salts, indicating an additional defect at the level of aminoimidazole precursors. The compound has been isolated by charcoal chromatography and has been tentatively identified as 5-amino-4-imidazole(N-succinylocarboxamide) ribotide (S-AICAR), previously implicated as a purine precursor by Lukens and Buchanan (Fed. Proc. 15:305, 1956). It contained an aminoimidazole (Pauly reaction) and ribose and phosphorus in equimolar amounts; yielded aspartic acid on acid hydrolysis; and showed an ultraviolet absorption spectrum with maximum at 267 mμ. It could be distinguished from 5-amino-4-imidazolecarboxamide ribotide (AICAR) by paper chromatography and by a peculiar instability in its behavior with the Bratton-Marshall reagents for diazotizable amines. Maximum color was obtained only when coupling was performed within 1 min after diazotization, yielding a purple pigment with maximum absorption at 560 mμ. Incubation of the compound with a yeast autolysate preparation containing high adenylosuccinase activity resulted in its conversion to AICAR. The results can best be explained by a single mutational event leading to the loss of 1 enzyme ("desuccinylase") required for 2 common functions; desuccinylation of both S-AICAR to AICAR and adenylosuccinic acid to adenylic acid.

807. COLLAGEN BIOSYNTHESIS. Bernard S. Gould and J. Frederick Woessner.* Div. of Biochemistry, Massachusetts Inst. of Technology, Cambridge.

The inability of scorbutic guinea pigs to synthesize collagen has been activated either to the inability to

form precursors or the inability to fibrilate some nonfibrous precursor of almost similar composition to collagen. Quantitative studies of skin regeneration in normal and scorbutic guinea pigs as well as on granulation tissue formed in subcutaneously implanted polyvinyl formaldehyde sponges, which serve as loci for collagen formation, have been made based on determinations of proline, hydroxyproline and glycine. Results show that if ascorbic acid is withdrawn at time of wounding considerable collagen forms even though no further ascorbic acid is administered. If withdrawn 4 days before wounding collagen formation is impaired and if withdrawn 7 days before, no collagen forms. This is notable since the half-life of administered ascorbic acid in such animals is 4 - 6 days. Depleted animals restored to ascorbic acid when wounded begin to deposit collagen only after 6 days. However, similar animals maintained on an ascorbic acid-free diet for 12 days after wounding and then given ascorbic acid produce collagen with 24 hr. In such animals the appearance of hydroxyproline, used as the measure of collagen, is accompanied by a concommitant decrease in noncollagenous proline and glycine of the granulation tissue suggestive that a pool rich in proline and glycine has been converted to collagen or to a precursor rich in hydroxyproline. Balance studies indicate that the reaction involves a quantitative conversion of proline to hydroxyproline. Preliminary fractionation indicates that the postulated precursor is of high molecular weight.

808. INFLUENCE OF THROMBIN ON THE RATE OF PROTHROMBIN CONVERSION. John L. Gray,* Donald G. Therriault* and H. Jensen. Army Med. Research Lab., Fort Knox, Ky.

Since it has been found that plasma accelerator globulin is associated with platelets, a phospholipid fraction from beef brain was employed as the thromboplastin source in an investigation of the influence of thrombin on the rate of prothrombin conversion. In order to obtain complete concersion of prothrombin to thrombin both antihemophilic factor (AHF) and plasma accelerator globulin (PAcG) must be present with phospholipid, serum eluate and $CaCl_2$. Serum eluate is a preparation obtained from aged rabbit serum by adsorption on $BaSO_4$ and partial purification of the citrate eluate. This preparation may not constitute a single entity and may contain proconvertin (factor VII, SPCA), plasma thromboplastin component (Christmas factor) and, perhaps, additional factors. It was found that traces of thrombin decreased the lag period which normally occurred before measurable prothrombin conversion began. Preincubation of AHF, PacG, serum eluate, phospholipid and $CaCl_2$ prior to the addition of prothrombin resulted in a marked enhancement in the prothrombin conversion. AHF and PAcG apparently will not interact with phospholipid and calcium ions in the absence of serum eluate. There was also no interaction of AHF and PAcG with serum eluate and calcium ions in the absence of phospholipid. Traces of thrombin in the preincubation mixture accelerated formation of the prothrombin conversion factor. The results indicate that the action of AHF and PAcG is potentiated by the presence of thrombin. Thus, a proper evaluation of a thromboplastin generation test should take into account the possible presence and amount of thrombin derived from prothrombin in the serum employed.

809. REDUCTION OF FERRITIN IRON BY XANTHINE OXIDASE. S. Green* and A. Mazur. Dept. of Medicine, Cornell Univ. Med. College and the New York Hosp., New York City.

Ferritin iron is reduced to the ferrous state by anaerobic incubation with liver slices. The reduced iron is dissociable and is released for transfer to iron-binding agents such as the iron-binding globulin of the plasma. An in vivo counterpart to this reaction occurs with liver hypoxia incident to hemorrhagic hypotension, during which we observed a marked increase in plasma iron and a corresponding decrease in iron-binding capacity. A study of rat liver slices indicates a relationship between reduction of ferritin iron and the conversion of hypoxanthine and xanthine to uric acid by xanthine oxidase; ferritin-ferric iron acts as an electron acceptor for the reduced enzyme and, in addition, can be reduced by the uric acid which accumulates. Lowered oxygen tension produces a marked increase in hypoxanthine, xanthine and uric acid in liver slices. In the absence of molecular oxygen, xanthine oxidase acts as a dehydrogenase and can maintain its production of uric acid from xanthine and hypoxanthine if ferritin iron is present. On the other hand, uricase of rat liver is completely inhibited by the absence of molecular oxygen, resulting in elevated values for uric acid. Confirmation of an alteration in purine catabolic reactions during in vivo tissue hypoxia is obtained from the elevated plasma uric acid values found in animals subjected to drastic hemorrhagic hypotension. These results suggest that changes in oxygen tension constitute one of the mechanisms regulating the release of hepatic storage iron to the plasma.

810. ESSENTIAL FATTY ACID AND PYRIDOXINE DEFICIENCY IN MONKEYS. Louis D. Greenberg and Henry D. Moon. Dept. of Pathology, Univ. of California School of Medicine, San Francisco.

In order to determine the effect of essential fatty acid (EFA) deficiency on the development of arteriosclerotic lesions in rhesus monkeys subjected to vitamin B_6 deficiency, single and combined deficiencies of EFA and B_6 are being studied in 10 young rhesus monkeys. The basal diet and supplements are essentially as described in our studies on B_6 deficiency (Am. J. Clin. Nutrition 4:318, 1956), except that in the study of EFA deficiency corn oil was omitted from the diet. The monkeys on the B_6-deficient regimen began to lose weight in about 3 wk, those on the regimen deficient in both B_6 and EFA began to lose weight after an average of 4 wk (2 animals in 2-3 wk and 1 after 7 wk) of deprivation, and of those deficient in EFA alone, 1 animal exhibited weight loss after 7 wk, but the other 2 continued to gain weight slowly. A reduction in activity and vigor was associated with weight loss in the first 2 groups. No other noteworthy changes in external appearance or behavior have occurred to date. Whole blood B_6 and transaminase levels which are lowered by B_6 deficiency were unaffected by EFA-deprivation. In several instances the excretion of xanthurenic acid following a test dose of 0.25 gm of L-tryptophan appeared to be greater on the doubly deficient regimen than on the one deficient only in B_6. (Supported in part by Grant A-645 from the Natl. Insts. of Health, PHS.)

811. CYTOCHROME OXIDASE IN THE LIVER OF THE RAT BEARING WALKER CARCINOMA 256. Alice Andrews Greene* and Frances L. Haven. Dept. of Biochemistry, Univ. of Rochester, Rochester, N. Y.

Both homogenates and mitochondria prepared from livers of normal rats and rats bearing Walker carcinoma 256 were assayed individually for cytochrome oxidase. The mean of cytochrome oxidase activities of the homogenates from 28 tumor-bearers was 136±4

μl O_2/hr/mg of dialyzed dry weight, and from 20 normals, 112 ± 4 μl O_2/hr/mg. The mean of cytochrome oxidase values of mitochondria isolated from 4 pairs of litter-mates was 555 ± 49 μl O_2/hr/mg for the tumor-bearers and 384 ± 33 μl O_2/hr/mg for the normals. The increase in cytochrome oxidase was a function of tumor size and became significant when the weight of the tumor reached 30% that of the total body weight. An increased cytochrome oxidase arising from essential fatty acid deficiency may be reduced to normal by correcting the deficiency (Tulpule and Williams. J. Biol. Chem. 217:229, 1955). The cytochrome oxidase of the tumor-bearers, however, remained elevated when the animals were given dietary supplements of essential fatty acids. (Supported by Research Grant C-669(C7) and Fellowship CF-5936 from the Natl. Cancer Inst., PHS.)

812. SOURCE OF THE THREE CARBON CHAIN OF SPERMIDINE. Ronald C. Greene (intr. by N. H. Horowitz). Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

Incorporation of radioactivity into spermidine was observed during growth of wild type Neurospora crassa in the presence of 2-C^{14}DL-methionine. The spermidine was isolated from a TCA extract of the mold and was further identified by comparison with authentic material in paper chromatographic and paper electrophoretic systems. The specific activity of the isolated spermidine shows that about 0.7 mole of labeled methionine was incorporated into 1 mole of spermidine. Similar levels of incorporation have been observed in a Neurospora methionineless mutant (strain 38706) blocked between homocysteine and methionine which indicates that incorporation does not proceed through reversal of the methionine synthetic pathway. Oxidation of spermidine with alkaline permanganate yields succinate (presumably from the 4 carbon chain) along with other products. Oxidation of synthetic spermidine labeled in the 4 carbon chain yielded radioactive succinate. Unlabeled succinate was obtained from the oxidation products of synthetic spermidine labeled in the 3 carbon chain and the spermidine isolated from Neurospora, indicating that the label from 2-C^{14} methionine is incorporated into the 3 carbon chain of spermidine.

813. CHARACTERIZATION OF SOME SULFATE ACCEPTOR ENZYMES. John D. Gregory and Yoshitsugu Nose.* Biochemical Research Lab., Massachusetts General Hosp., Boston.

The enzymatic transfer of sulfate from 3'-phosphoadenosine-5'-phosphosulfate (active sulfate, PAPS) to phenolic compounds and to steroids was identified with 2 separate fractions (sulfokinases) from soluble rabbit liver extracts. One produces the sulfates of steroids containing a β-hydroxyl group at carbon 3. The other sulfokinase transfers sulfate to many phenolic compounds, with a relative specificity similar to that of the detoxication reaction in whole animals. Estrogens of phenolic character are also sulfated by this fraction. As PAPS feeder for these reactions, the sulfate activating system of yeast or rabbit liver was used, or else p-nitrophenyl sulfate and 3'-phosphoadenosine-5'-phosphate (PAP) with liver phenol sulfokinase. PAP has been isolated from crude commercial ATP or ADP, of which it constitutes up to 4%. Phenyl sulfates as well as steroid sulfates may be estimated by the solvent-extractable methylene blue complex (Roy. Biochem. J. 63:294, 1956). The equilibrium constants and energy relationships of some of the transfer reactions have been studied and convenient assay methods have been developed for transfer enzymes, for PAP, and for acceptors. (Supported by grants from the American Cancer Society and the Life Insurance Med. Research Fund).

814. METABOLISM OF DIHYDROPYRIMIDINES AND RELATED COMPOUNDS. S. Grisolia, J. Caravaca,* S. Cardoso* and D. P. Wallach.* Dept. of Medicine, Univ. of Kansas Med. Ctr., Kansas City.

An enzyme catalyzing the interconversion of dihydropyrimidines and carbamyl amino acids has been purified 200-fold from beef liver acetone powder. This enzyme, about 80% pure, reacts with hydrouracil or hydrothymine or their corresponding carbamyl amino acids. The enzyme catalyzes also the conversion of hydantoin to carbamyl glycine but not the reverse reaction. We have also purified from the same source an enzyme which in the presence of $TPNH_2$ or TPN catalyzes the interconversion of pyrimidines and dihydropyrimidines. Substrates for this enzyme are uracil, thymine, iodouracil, bromouracil, hydrouracil and hydrothymine. An enzyme degrading carbamyl beta alanine to beta alanine CO_2 and ammonia has also been purified from supernatant fluids of rat liver homogenates. In addition, evidence for an enzyme which decarboxylates carbamyl aspartic acid to carbamyl beta alanine has been obtained. When dialyzed extracts of pigeon liver acetone powder are incubated with double carboxyl C^{14} labeled carbamyl aspartic acid, $C^{14}O_2$ is liberated and radioactive carbamyl beta alanine is formed as evidenced by paper chromatography using 2 solvent systems (isopropyl alcohol-water and phenol-citrate-phosphate).

815. ENZYMATIC DEFECT IN CONSTITUTIONAL NONHEMOLYTIC HYPERBILIRUBINEMIA (CNH) IN THE RAT. Gerold M. Grodsky and John V. Carbone (intr. by Harold A. Harper). Dept. of Medicine, Univ. of California School of Medicine, San Francisco.

Elevated levels of bilirubin in the serum of otherwise normal individuals have been described in premature infants and in adults (Gilbert's disease). A similar constitutional nonhemolytic hyperbilirubinemia (CNH) has been reported in a mutant strain of the Wistar albino rat. A metabolic disturbance in the hepatic mechanism for clearing bilirubin from the blood has been suggested as the responsible factor. Bilirubin is excreted from the body primarily as a conjugate of glucuronic acid, presumably through the following mechanism: glucose-1-phosphate $\xrightarrow{UTP}$ uridine diphosphate glucose $\xrightarrow{DPN}$ uridine diphosphate glucuronic acid (UDPGlu) + bilirubin $\longrightarrow$ bilirubin glucuronide. Previously, we demonstrated the conjugation of bilirubin in homogenates of liver and kidney from the normal rat. In the present study of homogenates of liver and kidney from CNH rats, no conjugation was observed, despite the presence of added bilirubin and UDPGlu. This suggests that the observed defect in bilirubin metabolism in CNH rats is caused, at least in part, by impaired activity of glucuronyl transferase, the enzyme responsible for the transfer of glucuronic acid from UDPGlu to the aglycone. No conjugation of o-aminophenol was observed in homogenates of liver from these animals, indicating that a generalized impairment of the mechanism of glucuronide conjugation, rather than a defect specific to the metabolism of bilirubin, is involved in constitutional nonhemolytic hyperbilirubinemia.

816. APPARATUS FOR CONTINUOUS RECORDING OF INSENSIBLE WEIGHT LOSS (IWL); CLINICAL AND EXPERIMENTAL APPLICATIONS. George M.

Guest and Mary D. Pettit.* Children's Hosp. Research Fndn., Cincinnati, Ohio.

For recording insensible weight loss (IWL), a simple apparatus has been devised employing 2 standard Toledo scales: 1 with 25-kg capacity (for infants and small animals), the other with 135-kg capacity, for adult human subjects. Weight is recorded electrically on paper pulled past the indicator needle of the scale and other needles adjusted to trace calibration lines. The indicator needle, swinging with movements of the subject, makes a tracing that gradually drifts across the paper with loss of weight. A line drawn through the tracing, crossing the calibration lines, permits close estimation of IWL as grams per hour, then expressed per kilogram of body weight and per square meter of body surface. The technique has been employed in studies of effects on IWL of metabolic stimulant and depressant drugs (e.g., thyroxine, dinitrophenol, thiouracil, barbiturates, etc.); effects on IWL of hyperventilation (e.g., with salicylism and diabetic acidosis); correlation of IWL with basal metabolic rates among patients with varying states of hyper- and hypothyroidism. (Studies aided by grants from Public Health Service No. A-306 and A-998.)

817. PYRIMIDINE MOIETY OF THIAMINE: ANTIMETABOLITE ACTION OF ANALOGS. Robert Guthrie, Maude E. Loebeck, Marilyn J. Hillman and Howard Tieckelmann (intr. by Christopher Carruthers). Roswell Park Mem. Inst. and Univ. of Buffalo, Buffalo, N. Y.

Among more than 200 pyrimidines tested for bacterial growth inhibition, several analogs of the pyrimidine moiety of thiamine, 2-methyl-4-amino-5-Xmethyl pyrimidine, were of particular interest because of increasee inhibitory effect upon amethopterin-resistant mutants of Bacillus subtilis and upon mutants of Escherichia coli resistant to purine antagonists when contrasted to the respective sensitive parent strains (Guthrie et al. Proc. Soc. Exper. Biol. & Med., in press). This collateral sensitivity to these compounds accompanying amethopterin-resistance appears related to the fact that in B. subtilis amethopterin growth-inhibition is effectively blocked by the thiamine pyrimidine, thiamine or thiamine pyrophosphate, (Guthrie et al. Bact. Proc., 1956). Eight analogs of the pyrimidine were obtained from others or prepared. Alkylmercapto, hydroxy or amino was substituted at the 2-position, while ethoxy, halogen, amino or hydroxy was attached to the 5-methyl carbon. All compounds produced growth-inhibition of B. subtilis specifically blocked by thiamine pyrimidine, thiamine or thiamine pyrophosphate. The most active antimetabolites, active at 10^{-7} M, possessed 2-methylmercapto and 5-hydroxymethyl or 5-halo-methyl groups. Four thiamine pyrimidine metabolites, differing in the group attached to the 5-methyl carbon, also varied in their effectiveness in preventing inhibition by the antimetabolite. 5-Hydroxymethyl was most active; 5-aminomethyl was least active. No such difference was obtained previously in blocking amethopterin growth-inhibition. These results are consistent with the hypothesis that amethopterin prevents synthesis of the thiamine pyrimidine from small precursors, while pyrimidine analogs interfere with its utilization.

818. TRYPSIN ACTIVATION OF A FERRICYANIDE LINKED PYRUVIC ACID OXIDATION. L. P. Hager (intr. by J. T. Edsall). Dept. of Chemistry, Harvard Univ., Cambridge, Mass.

In an investigation of the biochemical lesion in an acetate requiring mutant of Escherichia coli, it was found that crude extracts of the mutant catalyzed a rapid oxidation of pyruvate to acetate and CO_2 when ferricyanide served as electron acceptor. Purification of the crude extract disclosed a multiple enzyme requirement for this reaction. A soluble enzyme, obtained by ammonium sulfate precipitation, was stimulated 10-20 fold by the addition of mutant particles obtained by high speed centrifugation (144,000 x g) of the crude extract. During attempts to solubilize the particles with lipase, it was noted that crude pancreatic lipase preparations contained a soluble enzyme which replaced the mutant particles in the assay system. Subsequently, the replacing enzyme was identified as trypsin. Preincubation of the mutant soluble enzyme with varying levels of trypsin in the absence of ferricyanide followed by the addition of trypsin inhibitor duplicated the activation of the soluble enzyme by varying levels of mutant particles. In contrast to trypsin activation, particles activated the soluble enzyme only in the presence of the electron acceptor. Thiamine pyrophosphate is a required component of the trypsin preincubation mixture ($K_m = 6 \times 10^{-6}$ M) as well as the assay medium ($K_m = 1 \times 10^{-5}$ M). The nature of the trypsin activation and its relation to the particle activation of the soluble enzyme will be discussed.

819. GLYCOLYSIS AND LIPOGENESIS IN FETAL TISSUES DURING ANOXIA. D. D. Hagerman,* J. M. Loring,* D. B. Villee,* F. M. Wellington* and C. A. Villee. Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.

Fetal tissue metabolism was studied under aerobic and anaerobic conditions to evaluate the quantitative and qualitative differences in the metabolic pathways of fetal and adult tissues which might explain the greater ability of the fetus to survive hypoxia. Slices were incubated in phosphate buffer in an atmosphere of oxygen or nitrogen. The substrate, uniformly labeled glucose or fructose, glucose-1-C^{14}, glucose-6-C^{14}, pyruvate-1-C^{14}, pyruvate-2-C^{14} or acetate-2-C^{14}, was tipped in from the side arm after gassing. The amounts and specific activities of respiratory CO_2, tissue lipides and glycogen, and the glucose, fructose, pyruvate and lactate of the incubation medium were measured. There was no marked trend in the rate of lipogenesis by liver over the range of ages studied, 13-23 wk. Aerobic lipogenesis in human fetal liver at this stage is about 1/2 that of fetal rat liver just before term. Anaerobic lipogenesis from pyruvate, acetate, glucose or fructose in human fetal liver at this stage is only 10% of the aerobic rate. This is in marked contrast to rat liver just before term in which lipogenesis from pyruvate occurs at equal rates aerobically and anaerobically (Fed. Proc. 15:375, 1956). Lactate production is greater and lipogenesis is less under anaerobiosis. Human fetal heart, unlike liver, lung and brain, has equal rates of lipogenesis in oxygen and nitrogen. Under aerobic conditions the fetal liver metabolizes about half of the glucose via the pentose shunt. Fetal liver rapidly converts fructose to glucose. The hypothesis that some of these changes represent enzymic adaptations to hypoxia will be discussed.

820. 6-AMINONICOTINAMIDE AND RELATED NIACIN ANTAGONISTS. S. L. Halliday,* A. Sloboda,* L. W. Will* and J. J. Oleson. Exptl. Therapeutics Research Section, Research Div., American Cyanamid Co., Pearl River, N. Y.

The antivitamin and carcinostatic activity of 6-aminonicotinamide have been reported (Fed. Proc. 15:284, 1955; Proc. No. Am. Radiol. Soc., 1956). Further studies on the biological activity in rats and tumor-bearing mice will be presented. When the compound is fed to rats at levels of 15-30 mg/kg diet, niacin deficiency symptoms, including loss of motor control, paralysis, kyphosis, anorexia, diarrhea and bloody urine, are produced. These symptoms are prevented by 150-300 mg/kg of niacinamide in the diet. As a tumor inhinitor, the compound is active against 8C3HED lymphosarcoma, 755 and C3H mammary adenocarcinomas and relatively inactive against several other tumors. The tumor inhibition is reversed by niacinamide. 6-Aminonicotinic acid is about 1/7 as active as 6-aminonicotinamide. Comparative studies with other niacin antagonists, such as 2-amino-1,3,4-thiadiazole, pyrazine carboxamide, mercaptopyrazine and acetyl pyridine will be presented.

821. USE OF HYDRA FOR BIOCHEMICAL STUDIES ON REGENERATION. Richard G. Ham* and Robert E. Eakin. Clayton Fndn. for Research, Biochemical Inst. and Dept. of Chemistry, Univ. of Texas, Austin.

Quantitative methods requiring only 18 hr have been developed for measuring the rate of regeneration of hydra. Well nourished hydra which have been "decapitated" exhibit highly reproducible regeneration rates when maintained at constant temperature in chemically defined solutions containing all inorganic ions found to accelerate regeneration. Numerous biochemically active substances have been added to this controlled environment to determine their effects upon both the rate of regeneration and changes in the morphological structure of the regenerated organisms. Regeneration is retarded by numerous physiological inhibitors, including colchicine, nerve depressants, lithium salts and compounds known to interfere with protein and nucleic acid metabolism. Certain of these and other compounds also cause morphological alterations (e.g. an increase or decrease in the number of tentacles regenerated). On the basis that an agent exhibits activity only while the process it acts upon is operative, we have exposed regenerating hydra to specific types of agents for short intervals at various times during the regeneration period, thereby establishing the specific interval when each inhibitor shows a pronounced effect. Results indicate that very early there is a short period of rapid cell division, followed by a longer interval of reorganization during which the regeneration process is sensitive to nerve depressants; only subsequently do severed structures reappear visually. Thus, with these primitive organisms one can study biochemical mechanisms whereby multicellular organisms exert control over cell division and the organization of cells for morphological development.

822. CHROMATOGRAPHIC SEPARATION OF ACETONE SOLUBLE LIPIDES. Donald J. Hanahan and Edward J. Barron.* Dept. of Biochemistry, Univ. of Washington, Seattle.

The chromatographic separation on silicic acid columns of the acetone soluble lipides (essentially phospholipide free) of beef liver and bakers yeast has been investigated. As shown by Borgstrom (Acta physiol. Scandinav. 25:101, 111, 1952) using model lipide compounds and later by Fillerup and Mead (Proc. Soc. Exper. Biol. & Med. 83:574, 1953) employing pure compounds and also the plasma lipides, the various lipides can be separated on silicic acid by judicious choice of eluting solvent. In the present study a modification and extension of these observations, particularly with respect to solvent systems, has been made. When beef liver or bakers' yeast acetone soluble lipides are fractionated on a silicic acid column (18 mg lipide/gm silicic acid) with the indicated solvents the following components are eluted: a) hexane, pigments and hydrocarbons; b) hexane-benzene (8.5 - 1.5, v/v), sterol esters; c) hexane- 5% diethyl ether, triglycerides; d) hexane-20% diethyl ether, free sterols. The remaining components, comprised of free fatty acids, bile acids(?) and phospholipides, may be removed by 100% diethyl ether, 6% methanol-diethyl ether and 30% methanol-diethyl ether, respectively. The composition of the sources examined was: beef liver — sterol esters, 10%; triglycerides, 45%; free sterols, 30%; other lipides, 15%. Bakers' yeast — sterol esters, 3%; triglycerides, 44%; free sterols, 24%; other lipides, 29%. The details of this procedure will be discussed.

823. METABOLITES OF 6-AZAURACIL FORMED BY STREPTOCOCCUS FAECALIS. R. E. Handschumacher (intr. by A. D. Welch). Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.

Grown in the presence of the uracil analog, 6-azauracil (as-triazine-3,5-dione; AzU)-2-C^{14}, Streptococcus faecalis (8043) accumulated relatively large amounts of radioactive metabolites in the acid-soluble fraction of the cells. The degree of incorporation and the distribution of the radioactivity in the different metabolites were dependent upon the conditions of growth. Incorporation of AzU into the ribonucleic acids was only 1-5% of the amount found in the acid-soluble fraction; incorporation into the deoxyribonucleic acid was negligible. A major metabolite isolated from the acid-soluble fraction with the aid of Dowex-1 columns, and characterized as AzU-ribofuranoside, was indistinguishable from material prepared by a modification of the method used by Fox et al. for the synthesis of thymine riboside. In this synthesisa mercury-derivative of AzU was condensed with 1-chloro-tribenzoyl-ribofuranoside and the product was isolated after debenzoylation by ion-exchange chromatography. A second major metabolite in the acid-soluble fraction has been characterized tentatively as AzU-riboside-5'-phosphate; evidence concerning the nature of other phosphorylated derivatives will be presented. Certain AzU-resistant variants, which emerged from parent strains of microorganisms sensitive to AzU, were unable to utilize uracil-C^{14} and failed to form metabolites of AzU in significant amounts. However, some of these resistant strains utilized uridine and were strongly inhibited by AzU-riboside. It is suggested that in order to become an effective inhibitor of growth AzU must be converted to nucleoside derivatives.

824. REPLACEMENT OF METHIONINE BY HOMODJENKOLIC ACID IN GROWTH OF OCHROMONAS MALHEMANSIS. Hans J. Hansen (intr. by M. H. Hack). Dept. of Biochemistry and Lab. of Nutrition and Metabolism, Dept. of Medicine, Tulane Univ., New Orleans, La.

It has been shown (Miller and Yeager. Fed. Proc. 14:256, 1955) that djenkolic acid produced marked growth inhibition of Ochromonas malhemansis which was intensified in the presence of methionine. This effect suggested that a metabolite similar to djenkolic acid is involved in the metabolism of methionine by this organism. Homodjenkolic acid was synthesized and analyzed for carbon (found(F), 38.34; theoretical(T),

38.26); hydrogen (F, 6.27; T, 6.43); nitrogen(F, 9.88; T, 9.92); sulfur (F, 22, 75; T, 22.71) and oxygen by difference (F, 22.76; T, 22.67). After standing in alkaline NaCN, it gave negative tests for sulfhydryl groups. Infrared spectrophotometry shows it to be different from homocysteine, homocystine, djenkolic acid, S-hydroxymethyl homocysteine, cysteine, cystine and methionine. A solvent, acetone-butanol-cyclohexylamine-water, (10:16:2:5,v/v), was found which separated homodjenkolic acid from other sulfur amino acids on Whatman #1 paper. Inocula were grown on citrate salts media containing L-methionine (2 μm/ml) and vitamin B_{12} (0.1 mμg/ml). Experiments were run in the dark and growth measurements made daily for 10 days. DL-homodjenkolic acid (1 μm/ml) stimulated growth in the absence of B_{12} about 50% as much as L-methionine (2 μm/ml). However, in the presence of B_{12}, DL-homodjenkolic acid (4 μm/ml) stimulated growth twice as much as L-methionine (2 μm/ml). Inhibition of growth by djenkolic acid (1 μm/ml), in the presence of L-methionine (1 μm/ml) and B_{12} was completely overcome by homodjenkolic acid (2 μm/ml). S-hydroxymethyl homocysteine was found to inhibit growth either in the presence or absence of vitamin B_{12} and this inhibition could be reversed with methionine.

825. MUSCLE ACETYL PHOSPHATASE. Isaac Harary (intr. by J. Snoke). Radioisotope Service, VA Hosp., Long Beach, and Dept. of Physiological Chemistry, School of Medicine, Univ. of California, Los Angeles.

The presence of acetyl phosphatase in animal tissue, but at the same time the absence of other evidence of acetyl phosphate as a metabolite in these tissues suggests another role for this enzyme. Consequently its possible function as a general hydrolase of high energy phosphate compounds or as an acetyl or phosphate transferase was investigated. The enzyme was purified 800-fold from KCl extracts of horse muscle, representing a 10-fold purification over that previously reported. It is remarkably stable in acid and heat, with an isoelectric point near pH 8.0 and a pH optimum at 5.3. The Km for acetyl phosphate is 8×10^{-3}. It shows no metal requirement, nor is it inhibited by versene or α, α dipyridyl. Iodosobenzoate, iodoacetate and PCMB, do not inhibit indicating sulfhydril groups are not necessary for activity. Phosphate and pyrophosphate inhibit competitively. Acetyl phosphatase will not catalyze the acetylation by acetyl phosphate of a group of possible acceptors nor will it catalyze the phosphorylation of glucose or creatine. Moreover it will not catalyze an exchange between radioactive acetate or phosphate with acetyl phosphate in presence or absence of CoA or ATP. The enzyme does not hydrolyze ATP, ADP, pyrophosphate, acetyl-CoA, adenylacetate or phosphoglycerol. It does not contain transacetylase, acetothiokinase, myokinase or ribonuclease activities. These results together with a previous report on the ability to hydrolyze propionyl or butyryl phosphate indicates the enzyme is a group specific hydrolase of acyl phosphates without transferase activity.

826. INCORPORATION OF ADENINE-8-C^{14} AND P^{32} INTO RAT THYMUS AT VARIOUS INTERVALS AFTER 100 r X-IRRADIATION. Helen Harrington* and Paul Lavik. Western Reserve Univ. Atomic Energy Project, Cleveland, Ohio.

In a previous investigation (Arch. Biochem. & Biophys. 54:6, 1955), it was found that the 24-hr incorporation of P^{32} into DNA, of orotic acid-2-C^{14} into DNA pyrimidines, and of formate-C^{14} into DNA guanine of rat tymus was significantly inhibited by 100 r total body X-irradiation, while the incorporation of adenine-8-C^{14} into DNA purines was not reduced. The present study was carried out to determine whether similar results would be obtained if the analyses were made after shorter periods of incorporation. Sixteen male Wistar rats were given 100 r and were immediately injected, along with 16 control rats, with a solution containing both adenine-8-C^{14} and P^{32}. Groups of 4 control and 4 irradiated rats were killed at 3, 12, 24, and 48 hr after the injection, and the thymus nucleic acids were separated by salt extraction, alcohol precipitation, alkali incubation and acid precipitation. The acid-soluble, lipid, RNA and DNA fractions were then assayed for phosphorus content, ultraviolet absorption and radioactivity. P^{32} and C^{14} were differentiated by counting with and without a plastic filter. The incorporation of P^{32} into the acid-soluble and lipid fractions was not changed by irradiation, but its incorporation into RNA and DNA was reduced to about 60% of the control value 12-24 hr after irradiation. In contrast there was no significant reduction of adenine-C^{14} incorporation into either RNA or DNA at any of the intervals studied; an increased incorporation was observed at 24 and 48 hr.

827. BIOSYNTHESIS OF THIAMINE. Daniel L. Harris and Joseph Yavit.* Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

It has been previously shown (Harris. Arch. Biochem. and Biophys. 41:293, 1952; 60:35, 1956) that the pyridoximers and B_6-like compounds competitively prevent thiamine biosynthesis in Neurospora. It has further been proposed that, by virtue of their chemical and structural similarities to the pyrimidine precursor of thiamine, these B_6-like compounds acts as competitive substrates in the reaction(s) catalyzing the condensation of 2-CH_3-4-NH_2-5-CH_2OH-pyrimidine (PYR) and 4-CH_3-5-CH_2CH_2OH-thiazole (TZA). A detailed analysis of the structural specificity of the inhibitors suggests that derivatives of the B_6-like compounds (most plausibly the corresponding phosphates) are the true competitive substrates. This indirect evidence has led us to postulate the existence of the following reactions: PYR + ATP (?) ---> PYR-PHOS + ADP (?) (1) and PYR-PHOS + TZA ---> B_1 + P_i (2). Using a cell-free extract of baker's yeast we have obtained a preparation which catalyzes the synthesis of thiamine as measured by the thiochrome assay. If PYR-PHOS + TZA are used as substrates, the rate of B_1 biosynthesis is greater than if PYR + TZA are so used. Moreover, under the former conditions, B_1 biosynthesis begins immediately, whereas with PYR + TZA, a lag period of several hours occurs before B_1 biosynthesis is observed. Preliminary experiments further indicate that if Mylase, a commercial enzyme preparation rich in phosphatases, is added to the incubation mixture, thiamine synthesis from either PYR or PYR-PHOS is inhibited.

828. HYPERGLYCEMIC SUBSTANCES IN URINE AND IN SPLEEN. Benjamin Harrow, Philip Tomashefsky,* Tom T. Iriye* and Casimir Funk. City College of New York and Funk Fndn., New York City.

While a number of investigators (Houssay, Dingemanse, Doisy) and one of us (B. H.) have reported the presence of a hyperglycemic factor in urine, we now report that there is also a hyperglycemic factor in spleen (much more abundantly than in urine). The method of preparation which was the same both for urine and spleen (a spleen extract

which represents the aqueous phase after the butanol extraction of the tumor-inhibiting substance has been considerably modified, and involves adsorption on charcoal, elution with butanol and acetic acid, and successive precipitations and extractions with several organic solvents. Some of the most active fractions gave s strong ninhydrin tests; and since the factor acts only parenterally, there is the possibility that it may be a peptide-like compound. We have confirmed Pellegrini's claim and of others that cobaltous chloride itself is strongly hyperglycemic. However, the spleen extract precipitated with cobaltous chloride is more active than the salt alone or the spleen product tested separately. There is also some evidence. in 1 or 2 of the fractions, of a hypoglycemic factor. The factor in spleen might lend itself to tests in cases of clinical hypoglycemia, perhaps in certain acute mental disorders. The spleen, in general, might play a role in the etiology of diabetes, especially in conjunction with certain blood-sugar diminishing natural substances, to be reported on later.

829. MODIFICATION OF YTTRIUM METABOLISM IN MAN BY CHELATING AGENTS. Hiram Hart and Betty Rosoff (intr. by Harry Sobotka). Div. of Neoplastic Diseases, Montefiore Hosp., New York City.

Previous communications reported the effect of various chelating agents on the tissue distribution and excretion of radioactive rare earth isotopes in normal and in tumor bearing animals and in man. The effect of variation of the metal or of the chelating agent was described. The relationship of metal filterability to metal chelate stability as studied in vitro was also reported. Data will be shown that by selection of the proper chelating agents or suitable chelate to metal ratio the excretion of radioactive yttrium may be as low as ~5% or as high as ~100% in man. These studies were performed on the same subject in order to minimize biological variations. In each study approximately 1 mg of yttrium, labeled with Y^{90} and chelated to various polyaminoacid type chelating agents, was administered. The molar ratios of chelating agent to yttrium ranged from 1:1 to 1000:1. Preliminary calculations indicate that is is possible to relate the excretion values to the physical properties of the yttrium chelates by coupled linear first order differential equations although the significance of diffusion phenomena must be examined with extreme care. The effects of different levels of excess chelating agent can be approximately predicted by the mathematical formalism. The relation of this work to animal studies with rare earths, to in vitro investigations, to decontamination problems, and to the general theory of tracer analysis will be briefly discussed.

830. IS ATP A CATALYST OR AN ENERGY DONOR? Una L. Hart (intr. by I. S. Kleiner). New York Med. College, Flower and Fifth Ave. Hosp., New York City.

It is believed that the large negative free energy change involved in the hydrolysis of the high energy phosphate bond is utilizable for biological synthetic and mechanical work. As other authors (Gillespie et al. Nature, 171:1147, 1953) have pointed out, the thermodynamic function ΔF is not "free energy" but is in reality another way of expressing an equilibrium constant. However, assuming that the ΔF of a reaction is available for work, it is not useful if the reaction does not proceed at a reasonably rapid rate. Unfortunately, thermodynamics gives no information about the kinetics of a reaction. In general, a reaction having a high energy of activation will proceed slowly at ordinary temperatures. If a catalyst is added which forms a stabilized activated complex with the reactants, the activation energy will be lowered and the reaction will proceed at a faster rate until equilibrium is attained. The function of phosphorylation in metabolism may then be to reduce the energies of activation by stabilization of the corresponding intermediate complexes through chelation and inductive effects. It is known that without phosphorylation many metabolic reactions do not occur at an observable rate. The role of ATP in metabolism may be that of a catalyst which functions as a phosphorylating agent and not as an energy donor. It fits the classical definition of a catalyst because it is not used up in the reactions, but is regenerated. Furthermore, it affects the rate at which equilibrium is attained.

831. TOXIC ACTION OF OXYGEN ON CARBOHYDRATE METABOLISM IN VITRO. Niels Haugaard, Marilyn Hess* and Harold Itskovitz.* Dept. of Pharmacology, School of Medicine, Univ. of Penna., Philadelphia, Pa.

Rat heart homogenates fortified with KCl and DPN catalyze, at a rapid rate, the complete oxidation of glucose to CO_2 and H_2O. Oxygen uptake and glucose utilization by this multi-enzyme system was determined at different tensions of oxygen. Initially, the rates of oxygen uptake were almost the same in 7% oxygen, air or 100% oxygen. However, after about 1 hr of incubation of the system at 37°C activity declined progressively with time. The time of onset was earlier and the extent of the fall in activity greater the higher the concentration of oxygen. After about 2 hr the system in 100% oxygen had ceased to take up oxygen while activity was still present in air or at 7% oxygen. The degree of oxygen poisoning was found to be dependent on metal ions in the reaction medium. Cupric ions, in low concentrations, greatly increased oxygen toxicity. In the range of 5×10^{-6} M to 10^{-5} M these ions had no effect when the gas phase was air, but were highly inhibitory in the presence of 100% oxygen. The chelating agent ethylenediamine tetraacetic acid (EDTA) prevented completely the inhibition by added cupric ions. EDTA also prevented or greatly diminished the toxic action of oxygen in the absence of added cupric ions. The relation of these findings to the phenomenon of oxygen poisoning will be discussed.

832. ORIGIN OF THE CARBON ATOMS OF L-RHAMNOSE. George Hauser* and Manfred L. Karnovsky. Dept. of Biological Chemistry and the Biophysical Lab., Harvard Med. School, Boston, Mass.

Experiments reported previously demonstrated that carbon atoms 1, 3, 4 and 6 of the L-rhamnose moiety of the rhamnolipide produced by Pseudomonas aeruginosa are derived from the α-carbons of glycerol. Similarly, carbons 2 and 5 of L-rhamnose are derived from the β-carbon of glycerol. Further studies using one of the enantiomorphic forms of glycerol-1-C^{14}, namely D-glycerol-1-C^{14}, yielded L-rhamnose labeled predominantly in carbons 1 and 6. This indicates that glycerol undergoes reactions which maintain the uniqueness of each primary carbinol carbon during its conversion to L-rhamnose. When D-fructose-6-C^{14} was the sole carbon source, the L-rhamnose formed was labeled almost exclusively in carbon 6. This could indicate that 3-carbon compounds formed by cleavage of the fructose skeleton might not equilibrate before contributing to the carbon skeleton of L-rhamnose. Alternately, D-fructose might be converted

to L-rhamnose without cleavage. Rhamnolipide is also formed in fair yield when the sole carbon source is DL-propanediol-1, 2. When a mixture of glycerol-1-C^{14} and unlabeled propanediol served as substrate, it was found that these compounds contributed rhamnolipide carbon in proportion to their concentrations. Although glucose depresses rhamnolipide formation, trace amounts of glucose-6-C^{14} added to a glycerol medium during the period of maximal rhamnolipide biosynthesis yielded a product that was significantly labeled. The ability of resting cells of Pseudomonas aeruginosa to oxidize a number of possible intermediates in rhamnose metabolism will be discussed.

833. ON THE CHEMISTRY OF A PHYSIOLOGICAL INHIBITOR OF BLOOD CLOTTING. E. Hecht (intr. by Walter H. Seegers). State Univ., Utrecht, The Netherlands.

During a simultaneous study on the lipid activator of blood clotting and on the clot inhibiting effect of the sphingomyelins, sphingosine was discovered as a physiological inhibitor of blood clotting. The observations giving rise to this and some interesting reactions of sphingosine will be described. Although sphingosine alone inhibits very strongly the clotting of plasma, it is able to promote the clotting activity of the lipid activator. Under certain circumstances, there are clotting times obtained from 1/6 to 1/10 of the values which are to be reached with optimal concentrations of the lipid activator alone. Sphingosine is a natural inhibitor of the plasma and it is quite possible that it is in abnormally high concentration in certain hemorrhagic diseases such as hemophilia.

834. PROTEIN-BINDING OF 2-HYDROXY-5,6,7,8-TETRAHYDRO-NAPHTHALENE IN FORTIFIED RAT LIVER PREPARATIONS. Erich Hecker (intr. by J. A. Miller). McArdle Mem. Lab., Med. School, Univ. of Wisconsin, Madison.

The formation of a protein-bound derivative of estradiol in fortified rat liver preparations has been described (J. Biol. Chem. 210:249, 1954). In the attempt to evaluate rings A and B of estradiol in this process 2-hydroxy-5,6,7,8-tetrahydro-naphthalene-8-C^{14} has been prepared and studied as a model compound. In the presence of TPNH and oxygen rat liver homogenates form protein-bound derivatives of the model compound and estradiol to similar extents. The enzymatic process is catalyzed by the microsome fraction and is inhibited by 10^{-3} M p-chloromercuri-benzoate (84%), 10^{-3} M n-ethyl-maleimide (55%), 10^{-3} M 8-hydroxy-quinoline (52%) and 10^{-3} M o-phenanthroline (54%). Only weak inhibition was obtained with 10^{-3} M α, α-dipyridyl (20%) and 10^{-2} M KCN (5%). Fe^{++} could completely replace the requirement for TPNH. In the presence of Fe^{++} and oxygen, SH-inhibitors increase the amount of the protein-bound derivative formed in the microsomal system; the addition of cysteine decreased the yield of this product. The data suggest the formation of a highly reactive intermediate capable of combining with a number of acceptors. In order to test whether this involves a quinol intermediate, tetralin-p-quinol has been synthesized. The results of carrier experiments will be reported.

835. MECHANISM OF ACTION OF 5-FLUORO PYRIMIDINES. Charles Heidelberger, L. Bosch,* N. K. Chaudhuri,* and Peter B. Danneberg.* McArdle Mem. Lab., Med. School, Univ. of Wisconsin, Madison.

5-Fluorouracil (FU) and 5-fluoroorotic acid (FO) are potent inhibitors of the growth of a number of transplanted animal tumors, and the former drug is currently undergoing clinical evaluation in cancer patients (Proc. Am. Assoc. Cancer Res. 1957). 5-Fluorouracil-2-C^{14} has been synthesized and administered to mice bearing the Ehrlich ascites carcinoma. After 12 hr the nucleic acids from liver, spleen and ascites cells were collected. The combined nucleic acids were dissolved and reprecipitated to constant specific activity and dialyzed to remove adsorbed impurities. After perchloric acid hydrolysis, the pyrimidine bases were separated by ion-exchange chromatography. There was an insignificant radioactivity in cytosine, uracil and thymine, indicating that loss of fluorine did not occur. The majority of the C^{14} was shown by chromatography in 3 systems to be present as 5-fluorouracil, which is thus incorporated in all 3 tissues as such. The label in the nucleic acids was shown in other experiments to be present only in RNA. Of the urinary radioactivity, 30% was present as FU. The effects of FU and FO on the biosynthesis of nucleic acids in vivo and in vitro in the glycolyzing suspension of Ehrlich ascities cells (described by LePage. Cancer Research 13:178, 1953) are being investigated. In both systems and with both drugs there was a 95% inhibition of the incorporation of formate-C^{14} into the methyl group of nucleic acid thymine. Similar studies are being carried out using labeled orotic acid, uracil, and P^{32} in order to localize the block.

836. CROSS REACTIONS OF β-LINKED POLY-GLUCOSES IN TYPE VIII AND TYPE III ANTI-PNEUMOCOCCAL SERA. M. Heidelberger and P. A. Rebers.* Inst. of Microbiology, Rutgers, The State Univ., New Brunswick, N. J.

This long known instance of cross reactivity is due to the presence of multiple units of cellobiuronic acid in the specific polysaccharides (S VIII and S III) of the two types of pneumococci. Recent studies in J.K.N. Jones' laboratory have indicated that the structure of S VIII is (β-glucuronic acid-1$\rightarrow$4-β-glucose-1$\rightarrow$4-α-glucose-1$\rightarrow$4-α-galactose-1$\rightarrow$4)$_n$. The first 2 sugars constitute a cellobiuronic acid unit, the 2nd and 3rd are equivalent to cellobiose. From the quantitative theory of the precipitin reaction of Heidelberger and Kendall any polysaccharide with multiple cellobiose groups should cross-react in type VIII antipneumococcal sera. Barley and oat glucosans, in which part of the glucose is so constituted, precipitated about 10% of the antibody in a type VIII horse serum and a type VIII rabbit serum. Iles mannan, in which cellobiose units are linked to a mannose chain, precipitated somewhat less antibody. The fractions of antibody reactive with the polycellobioses are independent of the fraction which precipitates with S III, a polycellobiuronic acid. S III has the structure (β-glucuronic acid-1$\rightarrow$4-β-glucose-1$\rightarrow$3)$_n$. It therefore seemed possible that the multiple-β-glucose-1$\rightarrow$3-β-glucose-1$\rightarrow$4 linkages which are also present in barley and oat glucosans might suffice to cause precipitation in type III antisera. This was tested and found to occur. Quantitative data on the cross reactions will be presented.

837. TRANSFER OF RADIOACTIVITY BETWEEN PROTEIN FRACTIONS OF HEN OVIDUCT HOMOGENATE. Richard W. Hendler (intr. by Elbert A. Peterson). Natl. Heart Inst., Bethesda, Md.

Cell-debris obtained by gentle centrifugation of a homogenate of hen oviduct, after the tissue was previously incubated as a mince with radioactive amino

acid, has been shown to possess most of the incorporated radioactivity, most of the ribonucleic acid and highest rate of turnover with respect to the radioactive amino acid (Hendler. J. Biol. Chem. 223:831, 1956). This sediment also contains the microsomal material as determined by light and electron microscopic techniques (Hendler, Dalton and Glenner, in press). To test the possible precursor role of this fraction with respect to the other proteins of the cell, broken cell incubations were performed. It was found that free amino acids were entirely inert, but previously labeled cell-debris imparted radioactivity upon subsequent incubation, to the proteins of an unlabeled supernatant, obtained from fresh oviduct tissue. This transfer of radioactivity was stimulated by addition of a mixture of coenzyme A, guanosine triphosphate, adenosine triphosphate and phospho enolpyruvate; all appear to be required for stimulation. Incubation of prelabeled cell-debris in buffer releases a small amount of protein having higher specific activity than the starting cell-debris or soluble proteins at the end of the preincubation. It seems possible that this radioactive material coming from the cell-debris represents normal soluble proteins of hen oviduct and that it has been the most recently synthesized either before or during the mixed incubation. Protein chromatography is contemplated to determine if the newly released radioactive proteins are identical to the normally occurring soluble proteins of this tissue.

838. INTRACELLULAR PUTRESCINE: A CHARACTERISTIC OF GRAM-NEGATIVE MICROORGANISMS. Edward J. Herbst, Robert H. Weaver and Donald L. Keister (intr. by Marie A. Andersch). Dept. of Biochemistry, School of Medicine, Univ. of Maryland, Baltimore.

It has become apparent that the individual members of the Gram-positive and Gram-negative groups of microorganisms have in common not only their respective staining characteristics but also several physiological properties (Bartholomew and Mittwer. Bact. Rev. 16:1, 1952; Mitchell and Moyle. J. Gen. Microbiol. 10:544, 1954). We have analyzed 10 Gram-positive and 10 Gram-negative organisms for the putrescine complex of growth factors. The cellular amines were identified by a) bioautographic analysis utilizing Hemophilus parainfluenzae, b) paper electrophoresis, or c) the XE-64 column technique (Rosenthal and Tabor. J. Pharmacol. & Exper. Therap. 116:131, 1956). The Gram-negative bacteria contain putrescine and Escherichia coli also stores some spermidine. In addition to the storage of 0.04-0.1 μM of putrescine/mg of cells there is in some cases an excretion of growth factor activity into the culture medium. Most of the Gram-positive bacteria which have been analyzed store no detectable putrescine or polyamine nor do they excrete these compounds into the medium. In several Gram-positive organisms growth factor activity was detected but the level of storage was much lower than in Gram-negative bacteria. Bacillus subtilis and Bacillus cereus, which are classified as Gram-positive but have a tendency to stain Gram-variable, has a putrescine concentration comparable to the lowest levels observed in Gram-negative species. Intracellular putrescine is therefore a biochemical characteristic of Gram-negative microorganisms. The possible relationship of this finding to certain structural features of Gram-negative cells will be discussed.

839. A NUCLEOTIDE PHOSPHATASE DEPENDENT ON A REDUCING AGENT. Emery C. Herman, Jr., and Barbara E. Wright (intr. by Olaf Mickelsen). Natl. Heart Inst., Bethesda, Md.

An enzyme system has been studied in crude cell-free extracts of Clostridium sticklandii (H.F.) which is capable of cleaving most of the mono-, di- and triphosphates of the nucleosides adenine, inosine and uridine. The end products have been determined in the case of adenosine monophosphate (AMP), which is cleaved quantitatively to adenosine and orthophosphate. Pyridoxal phosphate, phosphoglyceric acid, ribose-5-phosphate and adenosine-3'-phosphoric acid are not cleaved. After extensive dialysis, activity of the nucleotide phosphatase was found to be dependent entirely upon a reducing agent, Mg^{++} and yeast extract. The most active reducing agent was tetrahydrofolic acid; others which could be used were glutathione, 1', 3'-dithiopropanol and ascorbic acid. Ferric iron replaced the Mg^{++} and yeast extract requirements and finally it was found that ferrous iron replaced all of the requirements. It remains to be determined whether the activity of Mg^{++} in the presence of reducing agent is due to an iron contamination. Reactants were used in these concentrations: 3.0 μM AMP, 4.0 μM $FeCl_2$ and 4 mg enzyme protein. Samples were incubated anaerobically in a volume of 0.4 ml with M/10 tris buffer pH 7.4. The reaction is not blocked by iodoacetate or p-chloromercuribenzoate in concentrations of 10^{-2} and 10^{-3} M, respectively. Experimentation is under way to purify and characterize this enzyme system further.

840. MACROMOLECULAR COMPOSITION OF THREE STRAIN OF STREPTOCOCCUS PYOGENES. Eugene L. Hess, Hutton D. Slade* and Isao Komine.* Rheumatic Fever Research Inst., Chicago, Ill.

Qualitative and quantitative information concerning the macromolecular composition of 3 strains of Streptococcus pyogenes (N19, B19 and 12RN) will be presented. Cells were fragmented by means of sonic radiation. Cell-free extracts were subjected to electrophoretic and chemical analyses. The composition of the total cell has been expressed in terms of electrophoretic, solubility and/or dialysis properties. In each strain more than 80%, dry weight, of the cell was soluble in water. The macromolecular composition, judged by methods of analysis employed, was qualitatively identical for all strains. Striking quantitative differences in composition were found. Certain components have been obtained relatively free from others, judged by electrophoretic properties, by control of exposure to sonic energy and by altering the solvent system used for extraction. Extracts have been subjected to chemical fractionation studies employing pH and ionic strength as variables. From the results of these studies it has been concluded that: 1) electrophoretic components found in cell-free extracts represent cellular entities and are not factitious products of sonic dissolution technique and 2) extracts lend themselves to chemical separation processes. Component 4, defined by electrophoretic mobility, has been separated in relatively pure state from strain B19. This component migrated as a single symmetrical peak which amounted to 90% of the areas of the pattern. Chemical analysis indicated that this component was probably a PNA type nucleoprotein, in which the nucleic acid amounted to approximately 7%.

841. RAPID METHOD FOR THE QUANTITATIVE ASSAY OF PROTEOLYTIC ENZYMES. G. P. Hess,* E. I. Ciaccio* and W. L. Nelson. Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.

An extremely useful and sensitive method has been developed which allows the accurate determination of

proteolytic enzyme concentration at 1/10 the level previously possible. The use of an insoluble protein dye complex as substrate permits simple separation by filtration of the enzymatically formed soluble hydrolytic products. The concentration of these dye-labeled products, determined spectrophotometrically, is directly proportional to enzyme concentration. Because of the specific reaction of Indigo carmine and Congored with the basic or acidic groups, respectively, of proteins a uniformly-labeled substrate is readily prepared. Indigo carmine fibrin, stable at pH 1-6, is a suitable substrate for pepsin and Congored fibrin, stable at pH 7-9.5, is a suitable substrate for enzymes with a pH optimum near 7.5, such as trypsin. The simplicity and sensitivity of the method are illustrated by the general procedure for enzyme assay. To flasks, containing 100-200 mg of substrate and 5 ml of an appropriate buffer, enzyme is added to give an enzyme concentration of 1-10 μg/ml. At appropriate time intervals aliquots are withdrawn, filtered to remove finely suspended particles and the concentration of the soluble products is determined spectrophotometrically at 620 mμ when the substrate is Indigo carmine fibrin and at 495 mμ when the substrate is Congored fibrin. The spectrophotometric readings are directly proportional to the enzyme concentration at constant time and to the time interval at constant enzyme concentration. (Supported by grants from the Natl. Insts. of Health, PHS.)

842. EFFECT OF METAL CATIONS ON ADENOSINETRIPHOSPHATASE ACTIVITY OF RAT BRAIN. Helen H. Hess* and Alfred Pope. McLean Hosp., Waverley, and Harvard Med. School, Boston, Mass.

Crystalline ATP freed of Na^+ and neutralized with Tris was incubated for 15 min at 37°C in the presence of comminuted microtome-prepared frozen sections of rat cerebral cortex. The incubation mixture consisted of 50-60 μg fresh weight of tissue in 60 μl of solution. The latter was buffered at pH 7.4 (0.1 M glycylglycine) and contained 0.005 M ATP, 0.005 M $MgCl_2$, and varying concentrations of NaCl and KCl, singly and combined over the range 1-155 mM/l. Liberated orthophosphate was determined by a microspectrophotometric adaptation of the Fiske-Subbarow method. Virtually no ATP hydrolysis occurred unless Mg^{++} was present, and the rate was maximal when ATP and Mg^{++} were in equimolar concentrations. The enzymatic hydrolysis of ATP was enhanced by the addition of small amounts of Na^+. When the Na^+ concentration was 0.010-0.015 M, the activity was 60% greater than with Mg^{++} alone. This stimulatory effect declined at higher Na^+ concentrations. Equivalent amounts of K^+ did not stimulate Mg^{++}-activated ATP hydrolysis and higher concentrations were inhibitory. However, when a physiological concentration of NaCl was present (0.145 M), the addition of 0.010 M KCl increased the rate of ATP hydrolysis to double that found with Mg^{++} alone. Substitution of Ca^{++} for Mg^{++} reduced ATPase activity by about 75% and produced a system unaffected by Na^+ and K^+ (Supported by Research Grant B-361, Natl. Inst. of Neurological Diseases and Blindness, PHS.)

843. STRUCTURE AND FUNCTION OF MITOCHONDRIA IN IRREVERSIBLE HEMORRHAGIC SHOCK. H. Hift and J. G. Strawitz (intr. by W. H. McShan). Cardiopulmonary Research Lab. and Dept. of Surgery, Univ. of Wisconsin Med. School, Madison.

Previous work from this laboratory has shown that heart mitochondria isolated from dogs in irreversible hemorrhagic shock were altered in structure when viewed under the phase contrast microscope and yielded low P/O ratios in Warburg experiments. The present report deals with further attempts at characterizing this abnormality. It was found that "shock" mitochondria undergo swelling and "crescent" formation less readily than the normal under a variety of in vitro conditions. Water, acid and dinitrophenol extracts tend to contain more proteinous, nitrogenous and UV absorbing material when compared to normal preparations. The phosphate picture is being investigated at present. Also in progress are electronmicroscope studies which may throw light on the internal structure of "shock" versus normal mitochondria.

844. AMINO ACID SEQUENCES IN RIBONUCLEASE. C. H. W. Hirs. Rockefeller Inst. for Med. Research, New York City.

The detailed arrangement of amino acid residues in 4 sequences, totaling 50 out of the 124 residues in oxidized ribonuclease, has been worked out thus far. Numbering from the amino-terminal end in the partial structural formula (Hirs, Stein and Moore. J. Biol. Chem. 221:151, 1956), the sequences established or confirmed are the following: Residues 1-10, Lys.Glu. Thr.Ala.Ala.Ala.Lys.Phe.Glu.Arg.; 26-39, .Cys-.Asp-NH_2.Glu-NH_2.Met.Met.Lys.Ser.Arg.Asp-NH_2.Leu.Thr. Lys.Asp.Arg.; 62-68, .Asp-NH_2.Val.Ala.Cys-.Lys.Asp-NH_2.Thr.; 86-104, .Glu.Ser.Thr.Ser.Gly.Lys.Tyr.Pro. Asp-NH_2.Ala.Cys-.Tyr.Lys.Thr.Thr.Asp-NH_2.GluNH$_2$ Ala.Lys. . The starting products for the sequence studies have been the 13 principal peptides formed by tryptic hydrolysis (Hirs, Moore and Stein. J. Biol. Chem. 219:623, 1956). The 9 smallest peptides (2-7 residues) can be studied directly; the 4 large segments (19-22 residues) were first split individually by chymotrypsin to give the peptides expected on the basis of the partial structural formula. The principal method used for determining the sequences has been the Edman degradation; after each cyclization step the composition of the remaining peptide has been determined quantitatively by ion exchange chromatography. The allocation of the amide groups was checked by enzymatic hydrolysis with leucine aminopeptidase or carboxypeptidase, followed by chromatographic demonstration of the presence of asparagine or glutamine. To derive the complete structural formula of the enzymatically active molecule, the results of the sequence studies on oxidized ribonuclease are to be combined with the knowledge of the position of the disulfide bridges now being obtained by Anfinsen and Ryle and in this laboratory (Spackman, Moore and Stein, see abstract).

845. INCORPORATION OF ACETATE INTO FAT BY HOMOGENATES OF MAMMARY TISSUE FROM LACTATING RATS. Philip F. Hirsch* and Paul L. Munson. Biological Research Labs. and Dept. of Pharmacology, School of Dental Medicine and Med. School, Harvard Univ., Boston, Mass.

Lactating rat mammary tissue, because of its vigorous metabolic activity in vitro in the form of slices and the absence of stored carbohydrates, was selected for a study of the role of carbohydrate metabolism in the synthesis of fat. Cell-free homogenates, prepared in the presence of glucose and nicotinamide according to the method of Bucher (J. Am. Chem. Soc. 75:498, 1953), and with ATP, fructose diphosphate, DPN, oxalacetate and hexokinase added as supplements incorporated acetate-1-C^{14} into the lipide fraction, although only to the extent of about 1% that of the mammary slice in terms of equivalent amounts of total N. Addition of the 100,000 g supernate of rat liver, itself capable under the same conditions of only

a very low order of lipide synthesis, to the full supplemented mammary homogenate increased the incorporation of acetate into lipides to approximately 35% that of the mammary slice (compared on the basis of total N in the mammary component). The radioactive lipides thus obtained passed through a column of Amberlite-IRA-400 and were therefore presumably glycerides rather than free fatty acids. In preliminary studies of the identity of the active factor in the liver supernate, it was found to be lost by boiling for 2 min or by dialysis against potassium phosphate buffer at pH 7.4, and not to be CoA.

846. INTERMEDIATE REACTIONS IN PROTEIN BIOSYNTHESIS. Mahlon B. Hoagland and Paul C. Zamecnik. J. C. Warren Labs. of the Huntington Hosp. of Harvard Univ., Massachusetts General Hosp., Boston.

The rat liver amino acid activating enzymes preparation (Hoagland, Keller and Zamecnik. J. Biol. Chem. 218:345, 1956) contains ribonucleic acid (S - RNA) which becomes labeled with C^{14}-leucine (or valine or glycine) upon incubation with the amino acid and ATP. The ATP requirement is specific and the amino acid labeling additive. S-RNA so labeled with leucine is nondialysable, charcoal and Dowex-1 adsorbable and has a sedimentation constant around 1.4. The C^{14}-leucine-RNA bond is acid stable and alkali labile, and does not exchange with free C^{12}-leucine. The ninhydrin-CO_2 reaction indicates no free C^{14}-leucine. When labeled S-RNA is incubated with undiluted hydroxylamine and chromatographed on paper, a spot corresponding to leucine hydroxamic acid contains all the radioactivity. The activating enzymes preparation labeled with C^{14}-leucine (and reprecipitated twice at pH 5.2 from dilute solution to remove free ATP and C^{14}-leucine) will transfer most of the bound C^{14}-leucine to protein upon subsequent incubation with microsomes and GTP. The requirement for GTP is apparently specific. In the ascites tumor in vivo incorporation system S-RNA becomes labeled with leucine more rapidly than does the protein of the microsomal ribonucleoprotein particles (the most rapidly labeled protein fraction in this system). These experiments suggest that incorporation of labeled amino acids into protein is indeed dependent on the activation system. It is further postulated that initial activation of amino acids by formation of an enzyme-bound acyl-AMP compound is followed by a transacylation to S-RNA. This latter reaction is ribonuclease sensitive, while the former is not. GTP mediates the transfer of activated amino acid to peptide linkage by a mechanism as yet unknown.

847. METABOLISM OF ACETONE-2-C^{14} IN THE INTACT RAT. Donald C. Hobbs, Robert J. Hill and Roger E. Koeppe (intr. by T. P. Nash, Jr.). Div. of Chemistry, Univ. of Tennessee, Memphis.

Glutamic acid, aspartic acid, alanine and serine have been isolated from the carcass and liver proteins of fed rats given an intraperitoneal injection of acetone-2-C^{14}. These compounds, as well as liver glycogen, were degraded and assayed for radioactivity. In contrast to carcass alanine, which was labeled predominately in carbon 2, carcass glutamate, aspartate and serine were labeled chiefly in the carboxyl positions. Much the same pattern was observed with the liver amino acids. However, liver aspartic and glutamic acids were found to have a greater proportion of their total activity located in the noncarboxyl portions of the molecule than did the corresponding carcass amino acids. The data support the postulate that acetone is metabolized to a triose and to "acetate" and "formate." (Sakami and Lafaye. J. Biol. Chem. 193:199, 1951; Rudney. J. Biol. Chem., 210:361, 1954). Whereas the labeling in alanine reflects primarily the triose route, that in glutamic acid is more indicative of the conversion to acetate. Some estimate of the quantitative significance of each pathway may be obtained by comparing these 2 molecules. The results will be contrasted with those obtained from similar experiments using pyruvate-2 and 3-C^{14}. (Supported by the Atomic Energy Comm.)

848. UTILIZATION OF HYDROGEN OF SUBSTRATES OF PYRIDINE-NUCLEOTIDE-LINKED DEHYDROGENASES IN REDUCTIVE BIOSYNTHESES. Henry D. Hoberman. Dept. of Biochemistry, Albert Einstein College of Medicine, Yeshiva Univ., New York City.

The demonstration by Vennesland and co-workers of direct transfer of hydrogen between substrates of pyridinenucleotide-linked dehydrogenases and pyridinenucleotides makes possible examination of the question of homogeneity of co-enzyme "pools" in tissues of intact animals. In the present study the following deuterium-labeled compounds were prepared in racemic form and administered to fasting rats: α-deuterolactate, α-deuteromalate, and β-deutero-β-hydroxybutyrate. D concentrations of glycogen, mixed fatty acids and cholesterol of liver and of body water were measured. In other experiments employing α-D-lactate the concentration of free glutamate of liver was determined. The D concentration of liver glycogen was found to be more than 10 times greater than that of body water after administering α-D-lactate but was not elevated after giving either α-D-malate or a mixture of normal lactate and β-D-β-hydroxybutyrate. The D concentration of liver glutamate of rats receiving α-D-lactate was approximately 20% of that of body water. In separate experiments employing D_2O the observed D concentration of liver glutamate also indicated introduction of 2 carbon-bound H atoms from body water. No D enrichment of liver fatty acids or cholesterol was observed in experiments employing D-labeled substrates. The present findings suggest that in intact liver the pyridinenucleotide "pool" is heterogeneous and therefore that the utilization of the H of a given substrate in reductive biosyntheses may be specific. (Supported by grants from the Natl. Science Fndn., PHS and the Sugar Research Fndn., Inc.)

849. THE α-GALACTOSIDASE OF AEROBACTER AEROGENES; ITS CHARACTERISTICS AND INDUCED SYNTHESIS. David S. Hogness and Edwin H. Battley (intr. by David H. Brown). Dept. of Microbiology, Washington Univ. School of Medicine, St. Louis, Mo.

As a first step in determining the mechanism by which α-galactosides induce the synthesis of both α- and β-galactosidase in E. coli and A. aerogenes, we have investigated the characteristics of α-galactosidase and the kinetics of its induced synthesis, there being little information available concerning this enzyme. A. aerogenes was used since all attempts to extract α-galactosidase from E. coli have failed although the enzyme activity can be measured in whole cells. α-Galactosidase has now been purified 150-fold from crude extracts of induced A. aerogenes. It is physically distinct from β-galactosidase in that the 2 activities are associated with different protein fractions. α-Galactosidase catalyzes the hydrolysis of melibiose, methyl-, ethyl-, phenyl- and o-nitrophenyl-α-D-galactosides, but not of ethyl-α-D-thiogalactoside, nor any of the β-galactosides tested.

This enzyme is inactivated by oxygen, iodoacetamide, p-chloromercuribenzoate and N-ethyl-maleimide. If inactivated by oxygen, it can be reactivated by thioglycolate, cysteine, sodium sulfide, sodium cyanide or 2-mercaptoethanol. Addition of any one of the above α-galactosides to an exponentially growing culture of A. aerogenes causes as much as a 100-fold increase in the rate of α-galactosidase synthesis. At saturating concentrations (5×10^{-3} M), the increase in enzyme activity per unit increase in bacterial mass is maximal immediately after adding the inducer and thereafter remains constant. Besides functioning as inducers of α-galactosidase, all of the above α-galactosides, except the ethyl-α-D-thiogalactoside, simultaneously induce the synthesis of β-galactosidase. D-Galactose induces both galactosidases. All of the β-galactosides tested (lactose, methyl-β-D-galactoside, methyl- and isopropyl-β-D-thiogalactosides) induce only β-galactosidase.

850. INCORPORATION OF MESO-INOSITOL-2-H^3 INTO THE PHOSPHOLIPIDS OF SLICES OF PANCREAS AND BRAIN CORTEX. Lowell E. Hokin and Mabel Hokin.* Pharmacology Dept., McGill University, Montreal, Canada.

Earlier studies showed that when protein secretion was stimulated in pancreas slices or when brain cortex slices were incubated with acetylcholine there was an increased rate of turnover of phosphate but not of glycerol in the phosphoinositides (Hokin and Hokin. Canad. J. Biochem. Physiol. 34:349, 1956). These observations predicted that the increased turnover of phosphate in phosphoinositides should be accompanied by an increased turnover of inositol. Pancreas slices and brain cortex slices were therefore incubated with inositol-2-H^3 under various conditions and the phospholipids isolated and counted at infinite thickness in a Geiger-type flow counter. When protein secretion was stimulated in pancreas slices with acetylcholine there was a 3- to 5-fold increase in the rate of incorporation of inositol-2-H^3 into the phospholipids. Suspension of brain cortex slices in contact with acetylcholine led to a 50-100% increase in the rate of incorporation of inositol-2-H^3 into the phospholipids. These observations bear out the prediction that stimulation of protein secretion in the pancreas or the addition of acetylcholine to brain cortex slices stimulates the splitting off and the reattachment of inositol phosphate in phosphoinositide. This turnover of inositol phosphate in phosphoinositide probably plays the role of a carrier in transport mechanisms. (Supported by a research grant from the United Cerebral Palsy Research and Educational Fndn., Inc.).

851. RELATION OF VITAMIN B_6 TO LOSS OF CELL CONSTITUENTS BY L. ARABINOSUS. Joseph T. Holden and Jane Holman (intr. by Daisy G. Simonsen). Biochemistry Dept., Med. Research Inst., City of Hope Med. Ctr., Duarte, Calif.

Previous studies have shown that a vitamin B_6 deficiency in L. arabinosus 17-5 reduces the ability of this organism to accumulate glutamic acid from buffers containing this amino acid and glucose (Holden and Roberts. Fed. Proc. 15:276, 1956). Efforts to account for this change have disclosed morphological and metabolic differences between vitamin B_6-adequate and -deficient cells which may be related to the difference in glutamic acid uptake. Washed cells of L. arabinosus suspended in buffer liberate substances having an absorption maximum in the region of 260 mμ. The liberation of these substances is elevated 3- to 10-fold in vitamin B_6-deficient cells, increasing with the severity of the deficiency. This leakage is more pronounced at 37° than at 4°, in buffers and saline than in water, and only slightly diminished by the presence of glucose. At least 75% of the ultraviolet-absorbing material, the nature of which will be discussed, is dialyzable even after a 5-min incubation of the cells in buffer. It is almost completely removed from solution by charcoal. Observations similar to those above have been made with vitamin B_6-deficient cells of S. faecalis.

852. PENTOSE FERMENTATION IN LACTOBACILLUS PENTOSUS. B. L. Horecker, E. C. Heath,* J. Hurwitz* and A. Ginsburg.* Natl. Insts. of Health, Bethesda, Md.

The fermentation of pentose by Lactobacillus pentosus involves a novel cleavage of D-xylulose 5-phosphate to yield acetyl phosphate and glyceraldehyde 3-phosphate. Inorganic phosphate, thiamine pyrophosphate and sulfhydryl compounds are required; the enzyme has been obtained in highly purified form and named phosphoketolase. It is present in cells grown on L-arabinose or D-xylose. With arsenate in place of phosphate the reaction product is acetate; however, the enzyme does not catalyze the arsenalysis of acetyl phosphate. The reaction goes to completion and is completely irreversible. Neither C^{14}-acetyl phosphate nor C^{14}-triose phosphate is incorporated into xylulose 5-phosphate. Thus no activated C_2 or C_3 intermediate is formed during the reaction. Phosphoketosale can be employed for the analysis of mixtures containing xylulose 5-phosphate since other pentose esters do not react. The K_M for xylulose 5-phosphate is about 5×10^{-4} M and for inorganic phosphate about 10^{-3} M. Crude extracts of L. pentosus obtained from cells grown on L-arabinose or D-xylose contain an active acetokinase and in the presence of ADP the reaction products are ATP and acetate. Acetokinase activity is very low or absent in extracts from glucose-grown cells, suggesting that this enzyme is formed adaptively in response to acetyl phosphate.

853. PARTIAL SYNTHESIS OF NEW BILE ACIDS. S. L. Hsia* and Sidney A. Thayer. Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.

Two new bile acids, acid I and acid II, which were isolated from the bile of rats, were found to be metabolites of chenodeoxycholic acid. Acid I is isomeric with hyocholic acid, a 3α,6,7-trihydroxycholanic acid. Acid II (m.p. 200°, $[\alpha]_D^{25} = +38 \pm 2$), although resistant to oxidation by periodic acid, was oxidized by chromic oxide and the product identified as 3-keto-6,7-secocholanic acid-6,7-dioic acid by isotopic dilution of its 2,4-dinitrophenylhydrazone with the authentic derivative obtained from hyocholic acid and acid I; thus, acid II is also a stereoisomer of 3α, 6,7-trihydroxycholanic acid. Partial synthesis of acid II was effected by acetolysis of methyl 3α-acetoxy-6,7-epoxycholanate (m.p. 173-175°, $[\alpha]_D^{25} = 31 \pm 2$); hydrolysis with methanolic KOH gave the free acid. Since scission of an ethylene oxide ring usually results in the diaxial trans glycol (Furst-Plattner rule), the tentative structure of acid II is 3α, 6β,7α-trihydroxycholanic acid. Partial synthesis of acid I was also accomplished by 3 different methods of hydroxylation of methyl 3α-acetoxy-Δ^6-cholenate (m.p. 123-125°; $[\alpha]_D^{25} = +67 \pm 2$). In addition, acid I was obtained after oxidation by chromic oxide of the

product obtained by acetolysis of methyl 3α-acetoxy-6,7-epoxycholanate, reduction of the carbonyl by Meerwein-Ponndorf reduction and subsequent hydrolysis.

854. ENZYMATIC DEACYLATION OF N^{10}-FORMYL TETRAHYDROFOLIC ACID. F. M. Huennekens. Dept. of Biochemistry, Univ. of Washington, Seattle.

From the stoichiometry of the hydroxymethyl tetrahydrofolic (hFH_4) dehydrogenase reaction, it is evident that beef liver preparations contain an accessory enzyme system, capable of deacylating the reaction product, N^{10}-formyl tetrahydrofolic acid ($f^{10}FH_4$), to regenerate free FH_4: $f^{10}FH_4 + H_2O$ formate + FH_4. Pigeon liver preparations must be supplemented with a deacylating factor, derived from beef liver, in order to carry out the overall concersion of serine to formate in the presence of catalytic amounts of FH_4. The deacylation may be followed spectrophotometrically by observing the disappearance of $f^{10}FH_4$ and the corresponding appearance of FH_4 (λ_{max} at 295 mμ). The substrate for the reaction, $f^{10}FH_4$, may be generated enzymatically from: 1) anhydroleucovorin (f^{5}-$^{10}FH_4$); 2) $f^{5}FH_4$ + ATP; 3) formate + ATP + FH_4; and 4) hFH_4. The product, FH_4, released from $f^{10}FH_4$, may be detected by the serine hydroxymethylase-hFH_4 dehydrogenase reaction, or by the formate activating system.

855. BIOSYNTHESIS OF RNA PYRIMIDINES. Robert B. Hurlbert and Harold O. Kammen (intr. by G. A. LePage). Dept. of Biochemistry, Univ. of Texas, M. D. Anderson Hosp. and Tumor Inst., Houston.

The interconversion of several pyrimidine compounds and their incorporation into RNA is being studied in tissue homogenates. The compounds investigated are orotic acid-6-C^{14} and uniformly-C^{14}-labeled uridine, cytidine, CMP and UMP. These are incubated with sucrose homogenates of the Novikoff transplantable hepatoma, rabbit bone marrow and thymus and rat liver preparations in the presence of HDP, ATP, $MgCl_2$, DPN and pH 8.5 buffer. The nucleic acids are prepared by extraction with hot sodium chloride. The acid-soluble fraction is also prepared and, after carrier additions, uridine, cytidine, UMP and CMP are isolated by ion-exchange and paper chromatography. Orotic acid, UMP, CMP and cytidine are incorporated into the RNA by these broken cell preparations when phosphorylative conditions are maintained. Under these conditions the formation of RNA cytosine from UMP or of RNA uracil from CMP is barely detectable. Likewise, there occurs very little interconversion of UMP and CMP on the acid-soluble level, even though these compounds are present as the triphosphates. Cytidine serves as a relatively good precursor of RNA cytosine and is more effective than CMP as a precursor of RNA uracil. When phosphorylation in the incubation is not kept at a high level the incorporations into RNA are greatly diminished but dephosphorylation of the nucleotides and interconversions of uridine and cytidine are favored. The data at present suggest that in animal tissues the cytosine compounds are partially derived from UMP via uridine and cytidine.

856. PHOSPHATE METABOLISMS OF EHRLICH'S ASCITES CARCINOMA CELLS. Kenneth Ibsen* and Ralph W. McKee. Dept. of Physiological Chemistry, Univ. of California Med. Ctr., Los Angeles.

The in vitro addition of small amounts of glucose (up to 1 mM) stimulate the oxygen consumption of Ehrlich's ascites tumor cells, while the addition of larger quantities of glucose inhibit oxygen utilization. This inhibition is partially prevented by the addition of inorganic phosphate (Cancer Res. 16:364, 1956). Sixty minutes incubation of the tumor cells in the presence of glucose indicates that the inorganic phosphorus decreases and the easily hydrolyzable phosphorus increases. Employing enzymatic and chromatographic methods there is an indication of an increase of the ATP during the incubation period, the initial amount being about 2.8×10^{-6} moles/ml of tumor cells. In addition there is a decrease in the relative amount of AMP compared to ADP and ATP, the ratios at 0 and 60 min being 0.20 : 0.20 : 1.0 and 0.5 : 0.25 : 1.0, respectively. In conformity with the observations of Acs and Straub (Doklady Akad. Nauk. S.S.S.R. 95:1021, 1954) was found that when the glucose is completely utilized there is a partial reversal of this process with a release of inorganic phosphate. During glucose utilization we also observed an increase in the phosphorus content of the trichloroacetic acid insoluble fraction. These studies are being pursued with additional measurements of the phosphate intermediates in the metabolic cycles in an effort to determine the reasons for inhibition of oxygen consumption in the presence of glucose.

857. INCORPORATION OF GLYCINE-1-C^{14} INTO PROTEINS AND NUCLEIC ACIDS OF LIVER AND HEPATOMA NUCLEI. J. Logan Irvin, Jean Rotherham,* Elinor Moore Irvin, and David J. Holbrook.* Dept. of Biochemistry, Univ. of North Carolina, Chapel Hill.

Glycine-1-C^{14} was injected into adult rats bearing transplanted hepatomas. Twenty hours thereafter, the nuclei of liver and hepatoma cells were isolated. The nuclei were extracted with 0.05 M sodium citrate, and from this extract were isolated a globulin fraction (Protein I), a histone (Histone I), a ribonucleic acid fraction (RNA I), and a deoxyribopolynucleotide (DNA I). Extraction of the nuclear residues with 1 M NaCl yielded, after additional steps, a deoxyribonucleic acid (DNA II), a histone (Histone II), and a ribonucleic acid (RNA II). There were obtained from the residues: DNA III, RNA III, Histone III, and a lipoprotein (Protein III). The fractions were found to be distinct and different by analyses for amino acids and purines. For both liver and hepatoma, the specific activities of the protein fractions (in terms of protein-glycine) were in the order: Protein III > Protein I > Histone III > Histone II > Histone I; the specific activities of the RNA fractions were in the order: RNA III > RNA II > RNA I; and those for the DNA fractions were in the order: DNA III > DNA II > DNA I. The ratios of the specific activities of various fractions of the tumor nuclei to corresponding fractions of the liver nuclei were equal to or less than 1 with the exception of the DNA and histone fractions, for which the ratios were greater than 1 in each case. This suggests that histones and DNA are associated functionally as well as physically and it provides support for Kacser's hypothesis for the structure of the gene. (Aided by a grant from the American Cancer Society.)

858. HEMOGLOBIN INTERMEDIATES. Harvey A. Itano and Elizabeth Robinson.* Natl. Insts. of Health, Bethesda, Md.

The sigmoid curve obtained in equilibrium measurements of the reaction between hemoglobin and O_2 has been attributed to the presence of 4 hemes in each hemoglobin molecule (Hb_4) and to the addition of

O_2 in 4 successive, interacting steps to form oxyhemoglobin ($(HbO_2)_4$). Since the successive reactions are rapidly reversible, the intermediates, $(HbO_2)Hb_3$, $(HbO_2)_2Hb_2$, and $(HbO_2)_3Hb$, have not been separated. In the present series of experiments, stable compounds that differ in charge were chosen for study. Oxidation of carbonmonoxyhemoglobin ($(HbCO)_4$) to ferrihemoglobin (Hb_4^{+4}) by ferricyanide occurs with release of CO and acquisition of a positive charge at acid pH by each heme oxidized. Partial oxidation of $(HbCO)_4$ results in electrophoretic heterogeneity demonstrable by moving boundary electrophoresis in phosphate buffer of pH 6.85, ionic strength 0.01. The components that appear upon partial oxidation migrate more rapidly as cations than $(HbCO)_4$ and more slowly than Hb_4^{+4}. By comparison of spectrophotometric and electrophoretic data, these components have been identified as intermediates in the successive oxidation of $(HbCO)_4$: $(HbCO)_4 \longrightarrow (HbCO)_3Hb^{+1} \longrightarrow (HbCO)_2Hb_2^{+2} \longrightarrow (HbCO)Hb_3^{+3} \longrightarrow Hb_4^{+4}$ (Itano and Robinson. J. Am. Chem. Soc., in press). These investigations have been extended to the reaction of Hb_4^{+4} and CN^- to form ferrihemoglobin cyanide ($(HbCN)_4$). $(HbCN)_4$ has the same electrophoretic mobility as $(HbCO)_4$. Partial conversion of Hb_4^{+4} to $(HbCN)_4$ is accompanied by the appearance of electrophoretic components of lower mobility than Hb_4^{+4} and higher mobility than $(HbCN)_4$. These components are intermediates in the addition of CN^- to Hb_4^{+4}: $Hb_4^{+4} \longrightarrow (HbCN)Hb_3^{+3} \longrightarrow (HbCN)_2Hb_2^{+2} \longrightarrow (HbCN)_3Hb^{+1} \longrightarrow (HbCN)_4$.

859. METABOLIC EFFECTS OF GLUCAGON IN DIABETICS. Joseph L. Izzo, Angela Roncone and Mary Ann Paliani (intr. by William S. McCann). Dept. of Medicine, Univ. of Rochester Med. Ctr., Rochester, N. Y.

Acute effects of glucagon particularly on blood sugar have been extensively investigated but little is known about metabolic responses to its prolonged administration. In the studies being reported effects of repeated daily administration of glucagon or organic and inorganic metabolism were determined in 3 stable and 3 unstable diabetics. Diabetics were selected for initial studies in order to reduce to a minimum possibility of obscuring responses to glucagon by secondary stimulation of pancreatic islets and also to evaluate importance of glucagon in diabetics. After suitable periods of sub-optimal and optimal control on chemically constant diets and insulin, 1 or 2 mg of highly purified amorphous glucagon (Lilly) was given every 8 hr intramuscularly for 3- to 9-day periods. Results were as follows: 1) variable but consistent rise in fasting blood sugar throughout entire glucagon period, the mean rise per patient ranging from 45 to 120 mg% above optimal control levels; 2) increased glycosuria in 5 patients ranging from 5 to 25 gm/24 hr; 3) uniform increase in urinary nitrogen, ranging from 12 to 34% above optimal control levels, with reversal of daily balance from positive to negative; 4) uniform absence of rise in urinary phosphorus comensurate with increased urinary nitrogen, and in 4 patients a concomitant slight inconstant fall in serum inorganic phosphorus; 5) no change in circulating eosinophiles but in 3 patients a consistent rise in daily excretion of urinary reducing corticosteroids ranging per patient from 40 to 75% above control levels; 6) no consistent changes in serum sodium and potassium or in daily sodium, potassium, and water balances. Possible role of glucagon in gluconeogenesis and interrelations with insulin and corticosteroids in metabolic homeostasis will be discussed in terms of these results.

(Supported by Public Health Service Grant A-611 and by a grant from Eli Lilly and Co.)

860. OXIDATIVE PHOSPHORYLATION BY RAT LIVER MITOCHONDRIA IN THE PRESENCE OF SIMPLE AND COMPLEX IONS. Earl Jacobs (intr. by M. Rothstein). Dept. of Physiological Chemistry, Univ. of California Med. School, Berkeley, and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

The oxicative phosphorylation system of rat liver mitochondria has a marked tendency to interact with simple and complex ions. Oxidation of ascorbate by these mitochondria can be mediated by the complex anions, silicomolybdate and ferricyanide. Coupled phosphorylation is obtained by additional use of Ba^{++}, Sr^{++}, Mn^{++} and higher levels of Mg^{++}. With ascorbate as electron donor, P/O ratios can be made to approach 3 by appropriate choice of complex anion, metal cation and ATP concentrations. P/O ratios greater than 1 are sensitive to low levels of antimycin. Silicomolybdate is bound by the mitochondria. This may reflect an interaction between the negative charges (- 4) on silicomolybdate and positively charged sites on the mitochondria. Bound silicomolybdate is apparently in a position to interact with the components of the electron transport chain. Silicomolybdate, bound to mitochondria, remains bound upon their exposure to distilled water, and mediates ascorbate oxidation as before, but phosphorylation coupled to electron transpot via cytochrome oxidase appears lost. With succinate as substrate P/O ratios are reduced to 1 by exposure to distilled water. This loss may reflect a change in the cation binding properties of the mitochondria. Examination of distilled water extracts revelas the presence of a hemoprotein which binds to anion exchange resins at pH 7. It is not yet known whether its presence in the soluble phase is related to the phosphorylation loss.

861. FORMATION OF CHOLESTEROL IN LIVER SLICES FROM BIOTIN DEFICIENT RATS. Gert M. Jacobsohn* and Ralph C. Corley. Dept. of Chemistry, Purdue Univ., Lafayette, Ind.

Cholesterol (sterols precipitable by digitonin) has been isolated from slices of liver from rats on complete and biotin-depleting diets after incubation with sodium acetate-2-C^{14} and dimethylacrylic acid-1-C^{14} (DMA). In comparison with the results in the control experiments, biotin deficiency has been found to be associated with a greater incorporation of radiocarbon from acetate but with a greatly reduced incorporation of radiocarbon from DMA into the sterol fractions isolated. Biotin does not appear to be necessary for the conversion of acetate to cholesterol. The increased conversion with lack of biotin may evidence a block in the metabolism of acetate by some other pathways. The results suggest that the utilization of DMA for synthesis of sterols is furthered by biotin and that carboxylation is a necessary step in the synthesis of sterols, as it apparently is in the metabolism of isovaleric acid (Fischer. Proc. Soc. Exper. Biol. & Med. 88:227, 1955). This view is generally consonant with the suggestion presented by Bloch (Essays in Biochemistry, ed. S. Graff, New York, 1956, p. 22) that DMA would be less likely to condense with itself than would, for example, methylglutaconate, and with the report of Tavormina, Gibbs and Huff (J. Am. Chem. Soc. 78:4498, 6210, 1956) that DL-β, γ-dihydroxy-β-methylvaleric acid is utilized to a very striking extent in the biosynthesis of cholesterol. (Supported by a grant from the Indiana Elks Assoc.)

862. AN ALDEHYDE DEHYDROGENASE REQUIRING INORGANIC PHOSPHATE. William B. Jakoby (intr. by Helen M. Dyer). NIH, Bethesda, Md.

A soluble pyridine nucleotide linked aldehyde dehydrogenase has been isolated from Pseudomonas fluorescence grown on ethylene glycol and has been partially purified. In the presence of phosphate and DPN the enzyme catalyzes the oxidation of a wide variety of aliphatic and aromatic aldehydes to their corresponding acids. The reaction is essentially irreversible. The phosphate requirement is fulfilled by arsenate; TPN is 20% as effective as DPN. The data suggest that the product of the oxidation is a free acid and not an acyl phosphate. Hydroxamic acid reacting material is not formed during the reaction nor are acetylphosphate and acetylglutathione cleaved in this system. Of the aldehydes tested only formaldehyde, chloral and glyoxylic acid are not oxidized. Of interest are a series of competitive reactions of aldehyde, DPN and phosphate with the enzyme.

863. CHEMICAL STRUCTURE OF β-HEPARIN. Roger W. Jeanloz, Pierre J. Stoffyn* and Monique Tremege.* Lovett Memorial Labs., Massachusetts General Hosp., and Dept. of Biological Chemistry, Harvard Med. School, Boston.

The chemical structure of the mucopolysaccharide β-heparin (Marbet and Winterstein. Helvet. chim. acta 34:2311, 1951), probably identical to K. Meyer's chondroitin sulfate B, has been investigated using methylation procedures. After six methylations with dimethyl sulfate and sodium hydroxide at 0-5°, a nondialysable substance was isolated (yield 75%) containing OCH_3 16.04%, N 2.68%, S 5.86%, $COCH_3$ 7.17%, Na 8.30%, in agreement with the values for a fully methylated polysaccharide. Scission by refluxing with MeOH-HCl 5% for 24 hr was followed by N-acetylation with acetic anhydride in methanol. Purification by chromatography on silicic acid gave three peaks in the approximate proportions 1:1:2, and with the following properties respectively: uronic acid (naphthoresorcinol): positive, negative, positive; nitrogen: negative, positive, positive. From the second peak crystalline methyl 2-acetamido-2-deoxy-6-0-methyl-α-D-galactopyranoside, m.p. 207-208°, was isolated, and further characterized by transformation into methyl 2-acetamido-2-deoxy-3,4-0-isopropylidene-6-0-methyl-α-D-galactopyranoside, m.p. 157-158°. In admixture with authentic products obtained by synthesis from D-galactosamine (Stoffyn and Jeanloz) neither m.p. was depressed. These data suggest that part or all of the galactosamine moiety is not substituted at position 6 and the glycuronidic linkage and sulfate ester are located at positions 3 and 4; their respective positions are as yet unknown.

864. IN VITRO EFFECT OF HYDROCORTISONE AND DESOXYCORTICOSTERONE ON GLUCOSE OXIDATION BY MESENTERIC LYMPH NODES AND LYMPHOSARCOMA OF THE RAT. Lillian A. Jedeikin (intr. by Harold J. Strecker). Dept. of Biochemistry, Albert Einstein College of Medicine, New York City.

Administration of certain 11-oxyadrenocortical steroids to a variety of animal species causes marked histological changes in lymphocytes in lymph nodes and thymus within 4 hr after steroid injection (Dougherty and White. Am. J. Anat. 77:81, 1945). Alterations in lymphocyte morphology have also been produced in vitro by addition of hydrocortisone. A basis for the lymphocytolytic action of hydrocortisone was sought in initial in vitro studies of the effect of this hormone and of desoxycorticosterone on oxidation of uniformly labeled C^{14} glucose by rat mesenteric lymph nodes. Whole nodes were incubated with C^{14}-labeled glucose in Warburg vessels at 37° with or without steroid. Respiratory $C^{14}O_2$ was absorbed in the center well by alkali soaked paper, the carbonate precipitated and radioactivity determined by standard techniques. With mesenteric lymph nodes from normal rats, in vitro addition of hydrocortisone produced inhibition of $C^{14}O_2$ production, with the degree of inhibition varying directly with incubation time and hormone concentration. No effect was observed on oxygen consumption. An even greater and more uniform inhibitory effect of hydrocortisone on $C^{14}O_2$ production was observed with mesenteric lymph nodes from adrenalectomized rats; with these tissues marked inhibition of oxygen consumption was also observed with hydrocortisone. Desoxycorticosterone added in vitro produced marked inhibition of $C^{14}O_2$ production and oxygen consumption of lymph nodes of adrenalectomized rats. Incubation studies with slices of Murphy-Sturm lymphosarcoma revealed a marked effect of added hydrocortisone in inhibiting $C^{14}O_2$ production and glucose utilization by this tumor.

865. EARLY EFFECTS OF ESTRADIOL ON NUCLEIC ACID METABOLISM IN THE RAT UTERUS. Kristian F. Jervell,* Carlos R. Diniz* and Gerald C. Mueller. McArdle Memorial Lab., Univ. of Wisconsin, Madison.

Surviving uterine segments removed from rats 6 hr following a single 10 μg dose of estradiol, incorporate one-carbon units derived from formate-C^{14}, glycine-2-C^{14} and serine-3-C^{14} into nucleic acid purines 3-5 times faster than control uteri (J. Biol. Chem. 219:585, 1956 and J. Biol. Chem. 223:369, 1956). Extension of these studies with $C^{14}O_2$ has demonstrated that the labeling of purines in positions other than 2 and 8 is also accelerated (2-3-fold); in contrast, the labeling of nucleic acid uridine was not affected by estradiol pretreatment. Analysis of the acid soluble fraction from uterine segments, incubated 1 hr with $C^{14}O_2$, revealed that the estrogen effect was primarily an acceleration of the incorporation into the acid soluble adenine nucleotides, which in turn was reflected in the labeling of the nucleic acid adenine. The accelerating effect of estradiol was demonstrable within 1 hr and increased linearly (approximately 300%) in the course of 6 hr. A similar response was observed on the labeling of acid soluble guanine. Quantitative nucleic acid analysis demonstrated that the RNA per uterus remained constant during this initial 6-hr induction period; this was followed by a rapid accumulation of RNA in the period between 6 and 24 hr. DNA content, as well as the labeling of thymine, remained constant throughout the first 24 hr. The segmental effect of estradiol on nucleotide metabolism in rat uteri will be discussed.

866. AN ACTIVITY OF NON-INFECTIOUS INFLUENZA VIRUS (PR8) PREPARATIONS. R. Bernal Johnson (intr. by W. W. Ackermann). Dept. of Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor.

In the continued study of phosphorus (P^{32}) incorporation into the individual nucleotides of the acid soluble fraction of viral infected cells, an enhanced rate of incorporation was detected as early as 30 min after infection. Further, the influenza virus appears to produce this stimulated rate of phosphorus incorporation into the chorioallantoic membrane when it lacks its full complement of biologic activities. Heat inactivated virus preparations stimulate phosphorus

incorporation as do active virus preparations, whereas virus inactivated by irradiation with ultraviolet light does not. Methionine sulfoximine, which has no effect upon phosphorus incorporation in uninfected membranes and inhibits viral replication completely, has only a partial effect upon the viral stimulated uptake of phosphorus. From concomitant investigations of the radioactivity of the intracellular pool of inorganic phosphate, it was concluded that the effects here are not upon the permeability of the limiting membrane of the cell. The stimulatory activity of other inactive preparations of virus and of active preparations which are in the presence of virus replication inhibitors will be discussed.

867. METABOLISM OF GROWTH FACTORS FOR RUMEN MICROORGANISMS: VALERIC ACID, VALINE, THE LEUCINES AND PROLINE. Ronald R. Johnson,* Burk A. Dehority,* O. G. Bentley* and Alvin L. Moxon. Dept. of Animal Science, Ohio Agr. Exp. Station, Wooster, Ohio.

Early in vitro investigations with rumen bacteria in mixed culture were performed using whole rumen liquor as an inoculum. Attempts to separate the bacteria from their mother liquor by centrifugation and washing have shown that previously unknown factors, necessary for their growth on a purified medium of cellulose, glucose, urea and minerals, were present in the mother liquor and in certain natural products. Valeric and caproic acids were identified as the stimulatory factors present in rumen liquor (J. Nutrition 57:389, 1955). By chromatography, the stimulatory factors in alfalfa extract, yeast extract and casein hydrolysate were shown to be a combination of the amino acids, valine or the leucines with proline. The vitamins biotin and para amino benzoic acid also are necessary. To study the metabolism of these growth factors, intermediates in postulated metabolic pathways have been substituted for the above factors. A combination of isobutyric or isovaleric acids with δ-amino-n-valeric acid gave activity similar to that given by a combination of valine or the leucines with proline. These results would conform to a pathway consisting of deamination of valine or the leucines to their respective α-keto acids and subsequent oxidative decarboxylation to the branch chain fatty acids. Simultaneously, the other factor, proline, might undergo ring cleavage to δ-amino-n-valeric acid. Carbon-14 labeled compounds have been used to further test these theories. When carboxyl-labeled valine is incubated in the in vitro rumen fermentation, considerable radioactivity appears in the CO_2 produced. This observation conforms to the proposed metabolic pathway, and attempts to isolate the labeled intermediates are now in process. Carboxyl labeled valeric acid has been tested to determine its relationship to the stimulatory amino acids. Very little of the C^{14} in valeric acid was liberated as CO_2 but much of it appeared to be converted to other volatile fatty acids and some was incorporated into saponifiable lipids.

868. TITRATION CURVES OF HUMAN EPIDERMIS IN RELATION TO AGE. Norman R. Joseph, Francois Bourliere* and Robert Molimard.* Lab. of Med. Biology, Univ. of Paris, Paris, France.

By an electrometric method it is possible to study the density of colloidal charge at the surface of human epidermis. This depends on the composition and state of aggregation of the colloids and on the pH of the skin. A curve showing the relation between charge density and pH is equivalent to a titration curve of the epidermis. Itotonic solutions of pH varying between 7.4 and 2.2 were used. Dilute phosphate buffers containing NaCl were used in the range pH 5.7 to 7.4, acetate buffers between pH 3.6 and 5.5. Below pH 3.1 isotonic mixtures of HCl and NaCl were used. The ionic strength of the solutions was 0.15. A group of 36 normal subjects between the ages of 5 and 95 was studied. In the growth period (age 5 to 20), the characteristic change was an increase of both acid and base-binding capacities of the skin accompanying a moderate increase of the isoelectric point to the adult level of pH 4.6. After age 20, the acid and base combining properties of the skin showed little change until about age 60. After the age of 70 there were decreases in acid-binding at pH 2.2, and in the density of groups dissociating between pH 2.2 and 5.0. These decreases were approximately proportional. Base-binding capacity at pH 7.4 showed no tendency to decrease. The isoelectric point became greater than pH 5.0 after age 80. From physicochemical and morphological considerations the changes in the titration curves are related to senescent diminution of keratin formation.

869. REDUCTION OF NEOTETRAZOLIUM BY SUCCINATE, ISOCITRATE, AND β-HYDROXYBUTYRATE IN WATER HOMOGENATES OF GUINEA PIG KIDNEY. Henry Kamin, Robert H. Gibbs,* and A. Donald Merritt.* Radioisotope Service, V.A. Hosp., and the Depts. of Biochemistry and Medicine, Duke Univ. School of Medicine, Durham, N. C.

In the course of studies of the defect in succinate oxidation in water homogenates of kidneys of tuberculous guinea pigs (Martin et al. J. Exper. Med. 101:639, 1955), using the neotetrazolium (NT) assay, it was observed that a heat-labile factor ("KS") in guinea pig or beef kidney water homogenates, sedimentable between 4000 and 12,000 x g, which did not itself catalyze NT reduction, stimulated NT reduction by succinate in dialyzed whole homogenate of normal guinea pig kidney. This factor could be replaced by cytochrome c. The nature of KS (microsomal cytochrome ?) has not been established. Dye reduction by succinate, and KS or cytochrome c stimulation, was abolished by antimycin A and cyanide. Unlike NT reduction by succinate, dye reduction by isocitrate was slightly inhibited by cytochrome c and the inhibition was relieved by cyanide and antimycin A. NT reduction by β-hydroxybutyrate was markedly inhibited by cytochrome c; inhibition was partially relieved by antimycin A and completely relieved by cyanide. Thus, in guinea pig kidney water homogenates in the presence of succinate, NT appears to be reduced at the cytochrome oxidase level of the electron transport system; with DPNH or TPNH (β-hydroxy butyrate or isocitrate) data are consistent with dye reduction below the antimycin-sensitive step.

870. IODO-INSULIN DEGRADATION STUDIES. Marvin Karasek* and Arne N. Wick. Scripps Clinic and Research Fndn., La Jolla, California.

Other workers (Williams, Mirsky) have provided much information on certain aspects of the metabolism of insulin I^{131} in intact animals and in isolated kidney and liver systems. Since insulin action is a striking one in the eviscerated-nephrectomized animal, which is a preparation assumed to represent predominantly muscle metabolism, we have used it to study the conditions that regulate the degradation of iodo-insulin. The rate of iodo-insulin degradation was measured by trichloracetic acid (TCA) precipitation. It was found that iodo-insulin is degraded at a slow and uniform rate after i.v. injection. One hour after the injection of iodo-insulin (0.5 unit kg) 25-30%

was degraded in contrast to the almost explosive rate (70-80%) observed in intact animals. The addition of carrier insulin (500 units kg) decreased the rate of degradation in the eviscerated animal to 8-15% in 1 hr with comparable lowering in rate in the succeeding hours. The rate of degradation is apparently not altered by the glucose load. Two extremes of glucose load were obtained by comparing the rate of degradation in animals maintained at a high blood sugar level and in other animals in which the glucose transfer rate was reduced to subnormal levels by the addition of 2-deoxyglucose. Since glucose load does not appear to alter the rate of iodo-insulin degradation, the significance of the iodo-insulin degradation observed in the absence of the kidneys and liver is open to speculation. Iodo-insulin was therefore treated with specific group reagents such as dinitrofluorobenzene (DNFB), phenylisocyanate, benzene diazonium chloride, formaldehyde, etc. The degradation rate of the iodo-insulin derivatives has been determined and compared with biological activity.

871. ENZYMATIC CONVERSION OF PHENYLALANINE TO TYROSINE. Seymour Kaufman. Lab. of Cellular Pharmacology - NIMH, Bethesda, Md.

The conversion of phenylalanine to tyrosine has been shown to require 2 protein fractions. Each of these fractions has been purified about 30-fold, one from rat liver and the other from sheep liver extracts (Kaufman. J. Biol. Chem. In press). The participation of more than one enzyme in this reaction has previously been reported by Mitoma (Arch. Biochem. 60: 476, 1956). With the partially purified enzymes, it has been shown that TPNH is the coenzyme involved; DPNH is considerably less active than TPNH. DPN^+ and TPN^+ are completely inactive. Balance studies support the following formulation of the reaction: $TPNH + H^+ + O_2$ + phenylalanine --- $TPN^+ + H_2O$ + tyrosine. The oxidation of TPNH by this system has been shown to be phenylalanine-dependent and, just as with tyrosine formation, requires the presence of both enzymes. The oxidation of TPNH can be followed spectrophotometrically and using this sensitive method, a distinct lag period has been demonstrated. This lag period can be completely eliminated by a short anaerobic preincubation of the sheep enzyme with TPNH (Kaufman. Biochim. et biophys. acta. In press); exposure to air leads to the reappearance of the lag. These results suggest that the first step in the reaction which leads to tyrosine formation may be the reduction of a cofactor by TPNH and that this step is catalyzed by the sheep enzyme.

872. THE ACTIVATION OF SUCCINIC DEHYDROGENASE. Edna B. Kearney and Thomas P. Singer. Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.

The activity of the succinic dehydrogenase of beef heart in soluble or particulate preparations, as measured at low temperatures (15°), can be increased if the enzyme is incubated with certain substances at higher temperatures prior to assay. Succinate, malonate, fumarate and phosphate all induce this activation of the enzyme to the same final degree (about 3-fold stimulation of activity), but very different concentrations of these compounds are needed to accomplish this. The effect of concentration of the activator on the rate of activation obeys Michaelis-Menten kinetics. Half-maximal activation is reached in the presence of 1.2×10^{-4} M succinate, 7.0×10^{-5} M malonate, 5.6×10^{-3} M fumarate and 0.11 M phosphate. The rate of activation follows first order kinetics, and the variation of the rate constants for activation with temperature indicates an activation energy for the process of 35,000 cal. per mole. The activation is not reversed by removal of the activating agent. The higher activity of this enzyme in phosphate than in other buffers (tris, glycylglycine, imidazole) reported earlier is probably due to the same activation phenomenon, since the concentrations of phosphate needed are about the same, the process is inhibited by arsenate and prior activation with succinate eliminates the need for phosphate in the assay. It is believed that these substances which combine with the active center induce a change in the protein, resulting in a higher turnover rate, altered Michaelis constants and changes in the absorption spectrum.

873. PYROPHOSPHATE FORMATION BY MICROSOMES. Francis T. Kenney and Elizabeth Barbehenn (intr. by E. V. McCollum). McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

The capacity for aerobic conversion of orthophosphate to pyrophosphate, exhibited by liver homogenates but not by respiring mitochondria, is largely restored to the latter by addition of the microsome fraction (Kenney and Colowick. Fed. Proc. 15:287, 1956). Nonoxidative ATP generating systems can be substituted for the mitochondrial fraction. The nonoxidative system used as a routine consists of phosphocreatine, the creatine kinase of rabbit skeletal muscle and catalytic amounts of ADP or ATP. With this system, as with the aerobic system, approximately 10-20% of the ATP phosphorus generated is converted by the microsomes to pyrophosphate, the remainder giving rise to orthophosphate. In contrast to generated ATP, added ATP gives rise almost entirely to orthophosphate, with little or no detectable pyrophosphate. CTP, GTP and UTP, when appropriately generated, appear to be equivalent to ATP as pyrophosphate precursors. For reasons which are not clear, orthophosphate is required for best yields of pyrophosphate from phosphocreatine, although experiments with P^{32}-labeled orthophosphate show clearly that it is not incorporated into pyrophosphate in the nonoxidative system. While ADP or ATP is essential in catalytic amounts (ca. 10^{-3} M) in the phosphocreatine system, either nucleotide is completely inhibitory at concentrations above 10^{-2} M.

874. CORRELATION OF GLUCOSE METABOLISM AND TRANSPORT WITH MUTAROTASE DISTRIBUTION AND KINETICS. A. S. Keston. N. Y. Univ. College of Medicine, New York City.

Keston (Science 120:355, 1954) proposed a unitary theory involving mutarotase, applicable to reabsorption of glucose by kidney and translocation of glucose into other tissues. The theory requires that mutarotase kinetics vary with glucose concentration in a manner similar to that known for glycolysis and reabsorption of glucose. Brain and kidney are known to be responsive to changes in glucose levels ca. 10^{-2} molar. Michaelis-Menten constants (K_m) for rat brain and human kidney mutarotases were calculated from measurements of mutarotation rates of alpha glucose. Measurements were made with photoelectric polarimeter (Keston. Abstr. Am. Chem. Soc., Cincinnati meeting, 1955) using water jacketed polarimeter tube. K_m values (by Lineweaver-Burk method) were: rat brain mutarotase, 4×10^{-2} molar (24°C); human kidney mutarotase, 3.2×10^{-2} molar (24°C), and 3.7×10^{-2} molar (30.6°C). An apparent K_m for the reabsorption of glucose by kidney of ca. 4×10^{-2} molar is obtained when the data of "glucose titration curve" (Smith, H. W. The Kidney. 92, Oxford Univ. Press, 1951) is plotted according to Lineweaver-Burk, replacing v by T/375.

Levels of mutarotase have been determined in various tissues. These levels are internally consistent for many tissues with published data for glucose utilization and reabsorption. Dog, man, rabbit and frog renal tubular maxima for glucose, divided by total units kidney mutarotase found (for unitage see Keston, Fed. Proc. 14:1, 1955) indicate correlation of one unit kidney mutarotase with reabsorption of about 3-6 mg glucose per minute (value for frog adjusted for temperature). Correlation of published glucose utilization values for some tissues having high glucose metabolism with their observed mutarotase content indicates ratios of the same order of magnitude as for kidney reabsorption. Typical values of mutarotase content per gram dry weight for such tissues are: retina (dog, frog, rat) of the order of .7-1 U; rat brain cortex, 1/4 U; rat embryo, 0.3 U; Jensen rat and Crabb hamster sarcomas, from 0.2 U to 1/3 U. Crystalline lens (bovine) contains high concentrations of mutarotase.

875. DIET AND SERUM CHOLESTEROL OF JAPANESE MEN IN JAPAN, HAWAII AND CALIFORNIA. Ancel Keys, Noboru Kimura,* Akira Kusukawa,* Brian Bronte-Stewart,* Nils P. Larsen* and Margaret Haney Keys.* Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.

Dietary studies on samples of Japanese men in Fukuoka Prefecture, Japan, showed that the percentage of calories provided by dietary fats ranged from an average of 9% among farmers to 22% among physicians. Samples of Japanese men in Hawaii averaged 32% and in Los Angeles 39% fat calories. Samples of Caucasian Americans in Minnesota, Los Angeles, Hawaii and Japan all averaged from 40 to 43% fat calories. The amount of linoleic and polyunsaturated fatty acids in the American diets was roughly twice as high as in the Japanese diets in Japan. The serum total cholesterol concentrations of these groups showed a close parallelism with the proportion of total calories supplied by total fats in the diet, ranging from an average of 141.5 ±3.0 among 50 Fukuoka farmers aged 40-49 to an average of 246.2 ± 5.8 among 45 Japanese men of the same age in Los Angeles. Within each occupation and age group the serum cholesterol concentration tended to rise with age from 20 to 60 years and there were indications of a decline at older ages. In all of the groups the amount of cholesterol in the alpha lipoprotein fraction of the serum tended to be constant, the differences in serum total cholesterol concentration being accounted for by the beta fraction. The serum cholesterol differences were not dependent on differences in average relative obesity or in physical activity between the groups. A dietary experiment on 18 coal miners in Japan showed that they responded as do Caucasian men in Minnesota to changes in the fat content of the diet.

876. ASSAY OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIC ACTIVITY IN BOVINE CORD FRACTIONS. Marian W. Kies, Elizabeth Roboz, and Ellsworth C. Alvord Jr. NIMH, Bethesda, Md.; Georgetown Univ. Med. School, Washington, D. C.; Baylor Univ. Med. School, Houston, Texas.

Encephalomyelitic activity of a bovine cord protein reported by Kies, Roboz and Alvord (Fed. Proc. 15: 1946) has been compared quantitatively with other cord fractions under standard assay conditions. Decreasing amounts of antigen in complete adjuvant were injected into guinea pigs (10 animals per dose) until the concentration range showing proportionality between dose and effect was reached. If the percentage of animals with obvious neurological damage is plotted against the logarithm of the dose, an approximate 50% effective dose (ED-50) can be determined graphically. ED-50 values determined in this manner for various fractions were: lyophilized whole cord, 0.07 mg; cord acetone powder, 0.04 mg; exhaustively defatted cord residue, 0.03 mg. The ED-50 of the glycoprotein fraction previously reported was 0.05 mg. This suggested attenuation of activity or loss of other more active fractions during isolation. Milder extraction procedures yielded a new fraction with an ED-50 of 0.004 mg, the most active encyphalomyelitic preparation yet reported. This fraction is nonlipid in nature, soluble in cold 5% KCl, represents 2% of the weight and approximately 20% of the activity of cord acetone powder. The results are in accordance with the theory that the compound in nerve tissue which induces encephalomyelitis in guinea pigs is protein rather than lipid in nature.

877. AMINO ACID SPECTRUM OF THE MATRIX OF URINARY CALCULI. J. Stanton King Jr. and William H. Boyce (intr. by William A. Wolff). Depts. of Biochemistry and Urology, The Bowman Gray School of Medicine, Winston-Salem, N. C.

Urinary calculi containing calcium salts or uric acid or cystine were decrystallized with Versene or veronal (pH 8.6) buffer and the residual organic matrix hydrolyzed (6N HCl, 90°C, 24 hr). The hydrolysates were chromatographed in two dimensions in each of three solvent systems. The following amino acids were present in all samples: α-alanine, glutamic acid, aspartic acid, serine, leucine, isoleucine, valine, phenylalanine, glycine, threonine, lysine and proline. These are arranged in the descending order of their relative intensities with ninhydrin. Tryptophane, tyrosine, methionine and arginine also appeared to be present but were faint and/or poorly separated. Microbiological confirmation was obtained for these results. At least two unidentified ninhydrin positive spots were consistently seen. The pattern of distribution was indistinguishable regardless of crystalline composition. A comparison of these results with those obtained from urinary proteins and salivary calculi will be presented. Urinary proteins were recovered by lyophilization of dialyzed urine and separated by ultrafiltration or preparative electrophoresis.

878. ISOLATION AND PROPERTIES OF GLUCOSE-6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES. Tsoo E. King and Vernon H. Cheldelin. Dept. of Chemistry and Science Research Inst., Oregon State College, Corvallis.

Crude cell free extracts of Acetobacter suboxydans can catalyze the oxidation of glucose through the pentose cycle. By combination of protamine precipitation, ammonium sulfate fractionation, calcium phosphate gel adsorption and column electrophoresis glucose-6-phosphate dehydrogenase was separated from 6-phosphogluconate dehydrogenase. The electrophoretic mobilities of these two enzymes were found to be very close both at pH 6.0 and 7.5. Isoelectric points of these dehydrogenases lay near pH 6.0. Preparations of highest activity catalyzed about 40 and 10 micromoles substrate per milligram of enzyme per minute, respectively, for the 6-phosphogluconate and glucose-6-phosphate dehydrogenases. For glucose-6-phosphate dehydrogenase, the turnover numbers with DPN and TPN were about equal, although at limiting concentrations of coenzyme, TPN was more effective. With 6-phosphogluconate dehydrogenase, DPN was much more effective than TPN. Other physicochemical characteristics will be reported.

(Supported by grants from the Nutrition Fndn. and the Natl. Insts. of Health, PHS.)

879. EFFECTS OF 17-HYDROXYCORTICOSTERONE ON CELL CONTENT CHANGES AND FORMATE-C^{14} INCORPORATION IN PROTEINS, PHOSPHOLIPIDS AND NUCLEIC ACIDS. David S. Kinnory, Roger Veatch, Joseph Greco and Eugene Kanabrocki (intr. by Alma Hiller). Radioisotope Service, V. A. Hosp., Hines, and Dept. of Biochemistry, Stritch School of Medicine, Loyola Univ., Chicago, Ill.

The involvement of hormones in the development and control of certain tumor growths lends particular interest to the mode of hormonal actions on cellular composition. The effects of 17-hydroxycorticosterone on the cell composition of mouse liver and small intestinal mucosa have been investigaged with respect to changes in concentration and incorporation of formate-C^{14} in cell proteins, phospholipids, and pentose- and deoxypentose nucleic acids. Fractionation and isolation of these cell components were accomplished by a modification of the procedure of Davidson and Smellie (Biochem. J. 52:599, 1952). These fractions were then chemically quantitated. A newly developed formamide-ethanol-toluene solvent system made it possible to measure the incorporation of C^{14} into these fractions by the more efficient internal liquid scintillation technique. Since the average DNA content in resting diploid nuclei is constant in all tissues of the same species (Boivin, Vendrely and Vendrely. Compt. redn. 226:1061, 1948), changes in cell composition were determined by relating the concentration of these cell constituents to that of deoxypentose nucleic acid in the same amount of tissue. The results of these investigations will be reported and the effects of 17-hydroxycorticosterone on the metabolism of these cell components will be discussed.

880. TPNH-CYTOCHROME C REDUCTASE AND NITRATE REDUCTASE FROM NEUROSPORA. Stephen C. Kinsky* and W. D. McElroy. McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

Highly purified preparations of the triphosphopyridine (TPNH) specific nitrate reductase (Nicholas and Nason. J. Biol. Chem. 211:183, 1954) have been obtained from the mold Neurospora crassa. Nitrate reduction is markedly stimulated by phosphate and the data suggest involvement of a phosphomolybdenum complex in the reaction. All the purified preparations of nitrate reductase contain an active TPNH-cytochrome c reductase which is not stimulated by phosphate nor inhibited by metal chelating agents. Either FMN or FAD are required for cytochrome reduction. DPNH is inactive as an electron donor. By determining cytochrome reductase and nitrate reductase activities at different purity levels and during heat inactivation, evidence has been obtained suggesting either the identity of these two enzymes or a common intermediate step. This conclusion is further substantiated by nutritional studies. Neurospora grown on ammonium chloride contain no nitrate reductase and 60% less cytochrome reductase than when grown on ammonium nitrate. Addition of minimal amounts of sodium nitrate to the basal growth medium induces the adaptive formation of both nitrate reductase and cytochrome c reductase activities. Additional evidence demonstrating the relationship between nitrate reductase and cytochrome c reductase will be discussed.

881. PARTIAL PURIFICATION OF RENAL GLUTAMINASE. Jack D. Klingman* and Philip Handler. Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.

The mechanism of action and metabolic significance of the phosphate-activated glutaminase of kidney have been established. As an initial step in such studies, purification of this enzyme was undertaken. A butanol powder of dog kidney was employed as starting material (Otey et al. Arch. Biochem. 49:247, 1954). The enzyme is readily extracted therefrom but thereafter the conventional procedures of enzymology resulted in complete loss of activity. If, however, extraction and all subsequent procedures are performed in 0.015 M borate, the enzyme remains stable during the purification process. Partial purification of the enzyme in the initial soluble extract of kidney powder has been achieved by the following manipulations: The borate extract is clarified by centrifugation, the protein precipitated by 1.6 M P_i, pH 8, redissolved in borate, inactive protein removed at 0.5 M P_i and the precipitate at 1.6 M p_i collected and redissolved. Inert protein is removed with safranin and the excess removed by passage over a Zeo Carb column. The protein is reprecipitated with P_i, redissolved, placed on an XE-97 column and eluted with a borate-glycine buffer. The last procedure is repeated yielding 80% of the original activity 40-80 fold purified. At any stage the enzyme may safely be dialyzed against 0.015 M borate but is inactivated by lyophilization. The powerful binding of glutamate exhibited by the initial extract is not shown by the purified enzyme which is, however, strongly inhibited by ammonia and by glutathione in concentration equimolar with substrate and by PCMB but not arsenite at 10^{-3} M.

882. METABOLISM OF C_{19} STEROIDS BY HOMOGENATES OF KIDNEY, HEART, TESTES AND PROSTATE. Charles D. Kochakian, Betty R. Carroll* and Barbara Uhri.* Oklahoma Med. Research Fndn., Oklahoma City.

Ten C_{19} steroids (testosterone; etiocholan-17β-ol, 3-one; androstan-17β-ol,3-one; epitestosterone; androsterone; epiandrosterone; dehydroepiandrosterone; 5-androstene-3β,17β-diol; androstane-3β, 17β-diol; androstane-3α,17β-diol) have been incubated with homogenates of kidney, heart, testes and prostate of male adult guinea pigs under conditions which had been previously shown to be optimum for the oxidation of the 17β-hydroxyl of testosterone by liver and kidney homogenates. The metabolites were isolated and characterized. A very wide variation not only in the metabolism of the respective steroids by the same tissues but also the different tissues was found. Kidney contained the greatest oxidase activity, prostate showed practically no ability to oxidize any of the steroids and epitestosterone was not metabolized by any of the tissues.

883. EVIDENCE FOR ADSORPTION AND PROTECTION OF UROKINASE BY WASHED HUMAN ERYTHROCYTES. S. N. Kolmen, M. M. Guest and D. R. Celander (intr. by Andrew A. Ormsby). Depts. of Physiology and Biochemistry, Univ. of Texas Med. Branch, Galveston, Texas.

Examination of human erythrocytes has revealed their ability to bind human urokinase. This property is enhanced if the erythrocytes are freed of plasma by washing with buffered saline. Solutions of urokinase exposed to washed erythrocytes decrease in potency and impart to the erythrocytes kinase activity. This phenomenon appears to occur most readily at pH 8. Although potent urokinase solutions added in vitro to plasma or whole blood are destroyed and elicit no fibrinolytic activity, washed erythrocytes, treated with buffered solutions of urokinase and washed again, elicit conversion of profibrinolysin to fibrinolysin when mixed in vitro with the plasma from which they were

taken. This conversion takes place even though evidence indicates that the amount of bound urokinase is exceedingly small. These observations suggest that erythrocyte-bound urokinase is protected from substances present in plasma which inhibit its action. Not only intact erythrocytes but also erythrocyte stroma adsorb urokinase. That some degree of stromatic organization is necessary is suggested by the observation that stroma, subjected to lyophilization, does not adsorb urokinase. In addition to urokinase, washed erythrocytes also adsorb inhibitors. Plasma treated repeatedly with washed erythrocytes steadily loses its insensitivity to urokinase. There seems little doubt that inhibitors to urokinase are removed. Whether inhibitors to fibrinolytic activity are also removed by adsorption on red cells is uncertain.

884. PHYSICOCHEMICAL PROPERTIES OF p-CARBOXY PHENYL AZO INSULINS. Walter L. Koltun (intr. by F. R. N. Gurd). Dept. of Biochemistry, Cornell Univ. Med. College, New York City.

The physicochemical properties of insulin coupled with p-carboxy phenyl diazonium sulfate have been studied by means of ultracentrifugation, solubility, paper electrophoresis and absorption spectrophotometry. The spectrophotometric and paper electrophoresis data indicate that all the diazonium salt is irreversibly bound and that the reaction occurs with tyrosine and histidine residues to form mono azo derivatives and with free amino groups to form bis azo derivatives. As more p-carboxy phenyl azo groups are attached there is a progressive shift and broadening of the region of minimum solubility to lower values of pH and a decreased solubility. In mildly alkaline solutions, where native insulin is in the form of dimers, trimers and tetramers, the increased electrostatic repulsive force between the insulin ions resulting from the introduction of 8.1 and 12.0 ionized carboxyl groups per 12,000 gm insulin, causes complete dissociation to the monomeric form (M = 12,000). The addition of 3.9 groups leads to an intermediate effect, i.e. shifts the equilibrium towards a greater proportion of monomers and dimers. The sedimentation patterns of azo insulins in acid solution show two peaks, a slow moving component which sediments like monomeric insulin, $S_{20,w} \sim 2.1$, and a fast moving component whose sedimentation coefficient increases as the ionic strength is raised or if more azo groups are attached but is relatively independent of protein concentration. It is suggested that the formation of soluble polymers results from the increased nonpolarity of the insulin molecule and that the factors limiting the polymer size may resemble those responsible for the formation of soap micelles.

885. ACTIVE TRANSPORT OF D-GALACTOSE-1-C^{14} BY SLICES OF RABBIT KIDNEY CORTEX IN VITRO. Stephen M. Krane* and Robert K. Crane, Dept. of Biochemistry, Washington Univ. Med. School, St. Louis, Mo.

The ability of slices of rabbit kidney cortex, in vitro, to concentrate D-galactose-1-C^{14} from the surrounding medium was observed by means of the following procedure. Slices were incubated, at 25°C under oxygen, in Krebs-Henseleit phosphate buffer, pH 7.4, to which D-galactose-1-C^{14} and raffinose had been added. At various time intervals, the tissue and medium were separated and a barium hydroxide-zinc sulfate filtrate of each was prepared. The galactose-1-C^{14} in the filtrates was diluted with nonradioactive galactose and converted to mucic acid-1-C^{14}, in which form it was recovered and assayed. Raffinose was measured colorimetrically. The tissue content of raffinose was used to correct the galactose-1-C^{14} assay for the amount present in the tissue extracellular space. Under the above conditions, tissue intracellular galactose-1-C^{14} increased with time to concentrations as much as three times greater than that of the medium at the end of 1 hr. The rate of this accumulation was concentration dependent and was inhibited by D-glucose but not by D-xylose. Replacement of oxygen with nitrogen or the addition of 3,5-dinitro-o-cresol prevented the accumulation of galactose-1-C^{14} to a level greater than that which represented, apparently, diffusion equilibrium and which was, numerically, 90% of the medium concentration. Phlorizin inhibited the rate at which diffusion equilibrium was attained and it reduced but it did not abolish the accumulation of galactose-1-C^{14}. The data are consistent with a hypothesis that phlorizin does not act primarily on the active transport process.

886. STOICHIOMETRY OF THE PHOSPHORYLASE b TO a REACTION. Edwin G. Krebs, Alan B. Kent,* and Edmond H. Fischer. Dept. of Biochemistry, Univ. of Washington, Seattle.

Rabbit skeletal muscle phosphorylase b has been isolated in high yields by a method involving its crystallization in the presence of Mg^{++}, AMP and cysteine. Crystallization also occurs in the presence of certain other divalent metal ions and mononucleotides. Under conditions promoting its crystallization the enzyme undergoes a dimerization readily observed in the ultracentrifuge. Phosphorylase b prepared in this manner was found to be ideally suited for a study of the stoichiometry of the b to a reaction (Biochim. et biophys. acta 20:150, 1956), since it was free of enzyme contaminants catalyzing undesirable side reactions. Using P^{32}-labeled ATP, it has been shown that the b to a reaction involving phosphorylation and dimerization of the protein may be formulated as: 2 phosphorylase b + 4ATP $\xrightarrow{E,\ Me^{++}}$ phosphorylase a-P_4 + 4ADP. No incorporation of the metal occurs as indicated by experiments using Mn^{54}. The phosphate incorporated into the phosphorylase a molecule is not removed by precipitation of the protein with TCA. It does not exchange with inorganic phosphate of the medium, when the enzyme catalyzes the phosphorolysis of glycogen. Contrary to the report of Engstrom and Agren (Acta chem. scandinav. 10:877, 1956), no incorporation of P^{32} was found to occur during the phosphorylase reaction, when nonlabeled enzyme is incubated with glycogen and labeled inorganic phosphate.

887. URINARY EXCRETION OF YTTRIUM CHELATES OF CYCLOHEXANE 1,2-TRANS DIAMINE TETRAACETIC ACID (CDTA) AND DIETHYLENETRIAMINE PENTAACETIC ACID (DTPA). Harry Kroll, Samuel Korman and Elsie Siegel (intr. by Georg. R. Cowgill). Olin Mathieson Chem. Corp., New Haven, Conn., and Div. of Neoplastic Diseases, Montefiore Hosp., New York City.

The removal of stable and radioactive elements from the human body is of considerable interest. The polyaminoacid types of chelating agents, of which ethylenediamine tetraacetic acid (EDTA) is best known, have played a prominent part in these studies, but despite the high affinity of EDTA for heavy metals, this chelating agent is unsatisfactory for detoxifying heavy metals when administered at low dose levels. In this report we present preliminary data on two chelating agents, cyclohexane 1,2-trans diamine tetraacetic acid (CDTA) and diethylenetriamine

pentaacetic acid (DTPA) which appear to be superior to EDTA for heavy metal detoxication. A series of human subjects were given intravenous injections of yttrium chelated with EDTA, CDTA and DTPA, respectively, and containing tracer amounts of Y^{90}. Urine samples were collected at 4, 8, 12 and 24 hr, and the excreted Y^{90} was determined in the urine. Yttrium as the CDTA and DTPA chelates was excreted in approximately 90-100% of the administered dose, whereas a similar dose of Y-EDTA was excreted at 50-60% levels. The higher urinary excretions of the yttrium chelates of CDTA and DTPA indicate that these two compounds would be more effective in removing rare earths from body tissues than EDTA. The interaction of yttrium chelates with the metal binding components of body tissues and fluids will be discussed.

888. INTERRELATIONSHIP OF DIETARY VITAMIN B_{12} OR FAT WITH THYROTOXICOSIS ON RESPIRATORY ENZYME ACTIVITY IN RAT LIVER. H. O. Kunkel and Wid P. Crawford.* Dept. of Biochemistry and Nutrition, Texas A. & M. College System, College Station.

Certain of the respiratory enzyme activities which are quantitatively increased as a consequence of thyrotoxicosis are also quantitatively altered by various dietary deficiencies. Cytochrome oxidase has been reported to be decreased in vitamin B_{12} insufficiency (O'Dell et al. J. Biol. Chem. 217:625, 1955) and increased in fat deficiency (J. Biol. Chem. 189:755, 1951). The reduction in liver cytochrome oxidase in growing rats receiving a B_{12} deficient diet was more severe in thyrotoxic rats than in rats receiving no thyroid substance. Preliminary studies indicated that the increases in cytochrome oxidase with thyrotoxicosis and with fat deficiency are not complementary. Thyrotoxicity did not increase the enzyme activity in fat-deficient rats. The simultaneous oxidation of citrate and malate by rat liver homogenates followed quantitative changes comparable to those exhibited by the cytochrome oxidase in fat deficiency as well as in thyrotoxicosis. The B_{12} deficiency had only a slight and nonsignificant effect on the citrate-malate oxidizing system. On a fresh tissue basis, thyrotoxicosis produced a diminution of DNA content in rats receiving the control and fat-deficient diets. In B_{12} deficient animals, however, thyrotoxicosis resulted in significantly augmented DNA content, so that when enzyme activities are related to DNA, presumably an indication of cell numbers, enzyme activities are not significantly altered when thyrotoxicity is imposed on B_{12} deficiency.

889. INHIBITION OF GROWTH OF E. COLI MUTANTS BY GALACTOSE. Kiyoshi Kurahashi and Albert J. Wahba (intr. by Herman M. Kalckar). NIH, Bethesda, Md.

Recently Schwarz et al. (Biochem. J. 62:34, 1956) observed an accumulation of galactose-1-phosphate in erythrocytes of galactosemic subjects. This accumulation of galactose-1-phosphate was found by Kalcker et al. (Biochim. et biophys. acta 20:262, 1956) to be due to a block in the step of galactose-1-phosphate uridyl transferase. By the use of methods for the determination of the activity of enzymes of galactose metabolism developed in this laboratory, it was found that E. coli K 12 galactose negative mutants described by Morse, Lederberg and Lederberg (Genetics 41:143 and 758, 1956) can be classified into two groups: one lacks galactokinase activity and the other galactose-1-phosphate uridyl transferase activity. The mutants which belong to the latter group were found to accumulate galactose-1-phosphate when they were grown in casein digest-yeast extract media containing galactose at 37°C. Furthermore, when these cells grown under the above conditions were inoculated into glucose synthetic media with and without addition of galactose, it was observed that the addition of galactose brought about a marked prolongation of the lag phase of growth of the cells. Slight, if any, effect of the addition of galactose on the growth of cells was observed with galactose positive cells and the mutants which lack galactokinase activity.

890. INFLUENCE OF FLUORIDE AND MAGNESIUM ON THE FORMATION OF BONE SALTS. Adrian C. Kuyper and K. Kutnerian.* Dept. of Physiological Chemistry, Wayne State Univ. College of Medicine, Detroit, Mich.

Inorganic salt solutions were prepared of composition similar to blood serum but saturated with carbon dioxide and with slightly increased calcium and phosphate concentrations. When these solutions were equilibrated with 6% carbon dioxide in air and adjusted to physiological pH and temperature calcium phosphate and carbonate were precipitated and within a few hours the levels of calcium and phosphate in the supernatant solutions became less than those of blood. When a small amount of magnesium was present in the precipitating solution, precipitation was initiated as before but ceased when the levels of phosphate and calcium in the supernatant solutions were about 5 and 9 mg %, respectively. This composition was maintained for several days as the solutions were equilibrated with 6% carbon dioxide in air, then precipitation began again and within several hours the levels of dissolved calcium and phosphate were reduced to low values. The rates of exchange of these precipitates with labeled calcium and phosphate indicate that the intermediate precipitate formed in the presence of magnesium dissolves and that a new, final precipitate is formed. Fluoride like magnesium stabilizes the intermediate precipitate but it is active only in the presence of magnesium. Fluoride decreases the solubility of the final precipitate when it is added to the supernatant solutions to give a concentration as low as 0.2 ppm. (Aided by Grant No. A613 from the Natl. Insts. of Health, PHS.)

891. FATTY INFILTRATION DUE TO RARE EARTHS. G. C. Kyker, E. A. Cress,* V. M. Sivaramakrishnan,* C. H. Steffee* and M. Stewart.* Med. Div., Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.

Following intravenous administration of various rare earths (3-4 mg/kg) to rats, their livers became blanched and distended. The gross appearance indicated acute fatty infiltration. Similar qualitative results were observed with a commercial mixture of rare earths and with 4 separate rare earths as follows: cerium, lanthanum, praseodymium and neodymium. Each was administered as a solution of the chloride at pH 3.5-4.0. The mixture and cerium caused more reaction than the others. The greatest damage was usually observed on the 3rd day. This pattern was seen by different methods. The oxygen consumption of liver slices was depressed to a minimum on the 2nd or 3rd day followed by gradual recovery. Fat-stained microscopic sections revealed a maximum of lipids on the 3rd or 4th day. The pattern of serum proteins as shown by paper electrophoresis was distinctly altered. Relatively, the amounts of alpha-1 and gamma globulins increased and albumin decreased. The maximum level of total lipids was from 3-4 times normal. Differences in the uptake of P^{32} by the livers at 1 and 8 hr are not clear but renal excretion at these intervals is greatly

elevated at 3 days after cerium. Other measurements revealed an increase both in the total weight of the livers and in their per cent of dry weight. The various measurements returned to approximately normal values by the 7th-10th day.

892. FORMATION OF A THIOETHER DERIVATIVE OF HOMOGENTISIC ACID CATALYZED BY HORSE RADISH PEROXIDASE (HRP). Bert N. La Du and Vincent G. Zannoni.* Lab. of Chem. Pharmacology, Natl. Heart Inst., Bethesda, Md.

It has been observed that HRP catalyzes the formation of phenolic thioethers. For example, in the presence of HRP these derivatives result from the interaction of homogentisic acid and various sulfhydryl compounds such as glutathione, cysteine or coenzyme A. The formation of these products does not require an exogenous source of hydrogen peroxide but is inhibited by catalase. Other heme proteins such as catalase, cytochrome c and hemoglobin cannot replace HRP. No reaction occurs in the absence of HRP or with boiled enzyme. Other phenolic substrates active in the formation of thioethers are hydroquinone, 2,6-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid and 2,5-dihydroxyphenylpyruvic acid. The products have the same U.V. absorption and fluorescence patterns as thioethers prepared chemically. Further studies are being made to determine whether these compounds are formed and can be degraded in vivo.

893. SYNTHESIS AND ACTIVITY FOR LACTOBACILLUS CASEI OF 6-ETHYL-(METHYL-)-7-METHYL-(ETHYL-)-9-(1'-D-RIBITYL)-ISOALLOXAZINES. John P. Lambooy. Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The appropriate substituted anilines were condensed with D-ribose and reduced to the ribitylanilines. The ribitylanilines were converted to the phenylazo compounds by the action of aniline diazonium chloride. The phenylazo compounds were reacted with barbituric acid to accomplish the unequivocal syntheses of 6-ethyl-7-methyl- and 6-methyl-7-ethyl-9-(1'-D-ribityl)-isoalloxazine. When either of these homologs of riboflavin is used to replace riboflavin in the well known microbiological procedure for the estimation of the vitamin, it is found to be a potent substitute for ribovlavin in the nutrition of Lactobacillus casei.

894. EFFECT OF OXYGEN ON LIPID SYNTHESIS. William E. M. Lands (intr. by Lila Miller). Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.

In lipid biosynthesis, fatty acids may be incorporated either into triglycerides or phospholipids, with D-α,β-diglycerides probably serving as intermediates for both process (Kennedy. J. Am. Chem. Soc. 78: 3550, 1956). The present experiments were designed to investigate factors which control the incorporation of fatty acids into the different lipids. Small amounts (2-3 gm) of slices or homogenized rat tissues were incubated with both P^{32}-phosphate and C^{14}-labeled precursors. The systems were buffered at pH 7.4 and incubated at 38°C in an atmosphere of controlled composition in a Dubnoff shaking incubator. Radioactivity from C^{14}-carboxy labeled acetate was incorporated to a greater extent into phospholipids than into phosphorus-free lipids when the incubation was carried out in an atmosphere of 95% oxygen, whereas the inverse was true for systems with less oxygen. Since acetate must be activated to a thioester and probably forms other fatty acid thioesters as intermediates in the pathway to either triglyceride or phospholipid, the high oxygen pressure is probably not needed for carboxyl group activation. The results suggest either that the oxygen supply may determine the type of glyceryl ester that is formed or that the diglycerides which form phospholipids do not equilibrate with those which form triglycerides. The small quantities of lipid involved in this type of experiment were separated most effectively by silicic acid columns which were developed. Micromolar quantities of lipids may be isolated from 1 to 5 gm of tissue by this method.

895. PREPARATION AND SOME PROPERTIES OF SOLUBLE GLUCOSE-6-PHOSPHATASE. Robert G. Langdon and David R. Weakley.* Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md.

It has been shown previously that a marked increase in the activity of hepatic glucose-6-phosphatase accompanies alloxan diabetes in the rat, and that this elevated activity is restored to normal levels by the administration of insulin. We have also found similar responses to occur in rat kidney and dog liver. It became of interest, therefore, to purify this enzyme and examine its properties. Although native glucose-6-phosphatase is tightly bound to the microsomal particles, it has been found possible to release the enzyme in soluble form by incubation of dog liver microsomes with digitonin. Partial purification of the soluble enzyme has been effected by ammonium sulfate fractionation. The partially purified enzyme has been found to be specific for glucose-6-phosphate; it has no activity on glucose-1-phosphate, fructose diphosphate, fructose-6-phosphate, betaglycerophosphate, or para-nitrophenyl phosphate. Maximal activity has been found at pH 6.0. The Michaelis constant has been found to be approximately 2.5×10^{-3} moles/liter. No metal or cofactor requirements have been demonstrated, although the enzyme is partially inhibited by fluoride ion in the presence of phosphate. The hydrolysis of glucose-6-phosphate is competitively inhibited by glucose, which has a K_I of 2.9×10^{-2} M/l. It is completely inhibited by 10^{-3} molar para-chloromercuribenzoate, and activity can be partially restored by glutathione. A number of chelating agents were found to have no effect on the enzymatic activity. (Supported in part by grant H-1731 from the Public Health Service.)

896. IDENTIFICATION OF THE SPECIFIC MILK PROTEINS SYNTHESIZED IN THE BOVINE MAMMARY GLAND BY C^{14} INCORPORATION. B. L. Larson and D. C. Gillespie (intr. by R. Jenness). Dept. of Dairy Science, Univ. of Illinois, Urbana.

Following an intravenous injection of 20 mc of C^{14}-sodium carbonate into a dairy cow, blood and milk samples were collected at intervals up to 24 hr and the individual component proteins isolated and analyzed for their C^{14} content. The high level of similar activity incorporated by α-casein, β-casein, α-lactalbumin and β-lactoglobulin indicates that these proteins are synthesized in close relationship to each other in the mammary gland from the same amino acid pool. The low level of similar activity incorporated by γ-casein, the immune globulins and milk serum albumin suggest that these proteins enter the gland in a preformed state; their similarities with the levels present in the blood proteins strongly suggest that they came from the blood stream. These proteins account for about 97% of the total proteins of skim milk and indicate that about 87% of the proteins present in normal bovine milk are synthesized from a free amino acid pool in the mammary gland.

897. SPECIFICITY OF DNase II FROM THYMUS. Ulla-Riitta Laurila* and M. Laskowski. Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

Deoxyribonuclease (DNase II) from thymus was prepared according to the method previously described, and was chromatographed on a CM-column. The enzyme thus obtained has a potency of about 3.0 (in previously described units). Deoxyribonucleic acid (DNA, 300 mg) was incubated with a large excess of enzyme for a period of 18 hr. The digest was chromatographed on Dowex 1-2X at pH 4.5. Mononucleotides accounted for about 8% of the total products and dinucleotides for about 3%. Each of the four mononucleotides was subjected to the action of 5'-nucleotidase from venom and was recovered unchanged, in about 90% yield, indicating that the mononucleotides were terminated in 3'-phosphate. Thus the specificity of DNase II from thymus resembles that of DNase from M. pyogenes and DNase from spleen. Five dinucleotides and one trinucleotide, all of which were terminated in 3'-phosphate, were isolated. The sequence of nucleoside residues was determined by the use of purified phosphodiesterase, which produces a nucleoside (from one end), a 5'-mononucleotide (from the center) and a 3', 5'-nucleoside diphosphate (from the other end). The dinucleotides were: CpCp, CpTp, ApTp, ApAp, GpAp, and the trinucleotide was GpApCp. No Pyp—Pup linkage was found so far among the isolated products. It is tentatively postulated that this linkage is preferentially split by DNase II. (Supported by the Atomic Energy Comm. and the American Cancer Society.)

898. REACTION OF FORMALDEHYDE WITH METHIONINE. Theodore F. Lavine, Norman F. Floyd* and Mary S. Cammaroti.* Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.

Formaldehyde reacts with methionine in acid solution to yield two main products, m-thiazane-4-carboxylic acid and the methyl sulfonium salt of methionine. The mechanism of this reaction is of interest because of possible bearing on the problem of the metabolism of one-carbon units. On the basis of indirect evidence, the methylol sulfonium salt of methionine, $RSCH(CH\ OH)^+X^-$, is postulated as the primary intermediate. Decomposition of the sulfonium function results in release of the methyl group and formation of the thiazane carboxylic acid; the methyl group combines with unreacted methionine to form the methyl sulfonium salt. Methionine methyl sulfonium bromide and m-thiazane-4-carboxylic acid were isolated in yields of 76% of the theory after heating equivalent amounts of methionine and formaldehyde in 12 N hydrochloric acid for 1 hr at 100°C. Other substances investigated as possible participants in the reaction included homocysteine, homocysteine thiolactone, homocystine and homodjenkolic acid. All of these yield the thiazane carboxylic acid by reaction with formaldehyde. Chemical properties that aided in characterization of the thiazane carboxylic acid were as follows: rapid oxidation to the sulfonic acid level by iodate in acid solution; rapid oxidation to the sulfone level by iodine in neutral solution; quantitative decomposition to homocysteine and formaldehyde by reaction with mercuric chloride under controlled conditions.

899. ACTIVITY OF GROWTH HORMONE PREPARATIONS ASSAYED IN ALKALI OR ACID. E. A. Lazo-Wasem* and Claire E. Graham. Wilson Labs., Chicago, Ill.

Two beef growth hormone preparations, one prepared by Dr. Li and one by Dr. Wilhelmi were compared for growth activity with Raben type pork growth hormone prepared at The Wilson Laboratories. The preparations were dissolved in water at pH 8 and at pH 4 and stored in the refrigerator for the 10-day test period. The beef growth hormone preparations produced superior growth in hypophysectomized rats when kept at pH 8, while the pork growth hormone was equivalent in activity at either pH. Both beef hormones were prepared by alkaline extraction, whereas the pork hormone was acid extracted. Beef growth hormone (Wilhelmi) maintained at pH 11.5 and made acid (pH 4.0) at the time of injection did not show reduced activity. This indicates that reduced activity results from storage at acid pH rather than destruction at the site of injection. A beef growth hormone preparation made at The Wilson Laboratories by the Raben procedure reacted like Raben pork growth hormone indicating the differential effect of pH to be due to the method of preparation rather than gland source.

900. INCORPORATION OF THE CARBON OF FORMALDEHYDE INTO LACTIC ACID BY PROPIONIBACTERIUM ARABINOSUM. F. W. Leaver (intr. by J. H. Jones). Dept. of Biochemistry, School of Veterinary Medicine, Univ. of Pennsylvania, Philadelphia.

It has been previously reported that the tracer of $HC^{14}HO$ is incorporated into all carbons of propionate in the propionic acid fermentation (J. Am. Chem. Soc. 74:2404, 1952). It was of interest to determine if the tracer of $HC^{14}HO$ also appears in the lactic acid. Resting cells were allowed to ferment 0.05 M lactic acid in the presence of 1.3×10^{-4} M $HC^{14}HO$ (500,000 cpm). At the end of 2 hr the fermentation was stopped by adding 10 N H_2SO_4. The bacteria were centrifuged down and the supernatant ether extracted. The volatile acids were removed by steam distillation. The lactic acid was recovered as the S-benzyl thiouronium salt and recrystallized to constant specific activity. The free lactic acid was regenerated and degraded. It was found that all positions of the lactic acid were labeled. The 3 carbon consistently had a higher specific activity than the 2 carbon. The possibility that the activity in the center carbon was due to partial randomization was investigated by fermenting lactic acid-3-C^{14} with resting cells under identical conditions. The unfermented lactic acid was then reisolated. It was found that essentially all of the tracer was still in the 3 carbon, indicating little or no randomization. Hence it appears that the formaldehyde carbon is incorporated into the fermentation before the formation of lactic acid and that extensive rearrangement of the carbon skeleton of the compound into which it is incorporated occurs before the lactic acid stage.

901. MICROSPECTROPHOTOMETRIC DETERMINATION OF INOSITOL IN TISSUES AND LIPIDE HYDROLYSATES. F. N. LeBaron,* J. Folch and E. E. Rothleder.* McLean Hosp., Waverley, and Harvard Med. School, Boston, Mass.

It has been found that, despite its high boiling point (290°), glycerol evaporates along with water from sufficiently dilute aqueous solutions. Under proper conditions, up to 10 mg of glycerol in 10 ml can thus be completely eliminated. This has made possible the separation of glycerol from inositol on a micro scale and the development of a microchemical method for estimation of inositol which has been applied successfully to lipides and brain tissue. The method consists of liberating inositol quantitatively and estimating the amount present by its reaction with

HIO_4 after complete removal of glycerol, carbohydrates, serine, ethanolamine and other interfering HIO_4-reacting substances. The sample, containing 10-100 γ inositol, is hydrolyzed by 6 N HCl in a sealed tube at 109° (refluxing 6 N HCl) for 40 hr. This destroys all carbohydrates and liberates inositol quantitatively. The hydrolysate is filtered, the filtrate dried to remove HCl, the residue dissolved in water and the solution treated once with Dowex-1 and twice with Dowex-50. These resins quantitatively remove traces of periodate-reacting breakdown products of carbohydrates, amino acids and amines, and strong acid anions which would cause some destruction of inositol during subsequent drying. Aliquots containing 2-20 γ inositol are taken, dried twice under infrared lamps from a volume of 10 ml to remove all glycerol, and the resulting residue treated with 0.5 ml 0.002 M HIO_4 for 90 min at 50°. The reaction mixture is diluted 24-fold and the remaining HIO_4 measured spectrophotometrically at 224 mμ. (Aided by Public Health Service Grant B-572.)

902. CRYSTALLINE 5'-ADENYLIC ACID DEAMINASE. Ya-pin Lee (intr. by W. H. Peterson). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

5'-Adenylic acid deaminase has been purified 800-fold from rabbit skeletal muscle and crystallized. It satisfies the following criteria of homogeneity: electrophoresis, sedimentation, diffusion, solubility, chromatography and enzymatic purity. The isoelectric point is approximately pH 5.6. The protein contains 16.5% of nitrogen. At 280 mu, the $E^{1\%}_{1\ cm}$ = 16.2 (potassium phosphate buffer, pH 8.0 or pH 6.4), and the ratio of extinctions at 280/260 were 1.12 at pH 8.0 and 1.11 at pH 6.4. The enzymatic activity was quite stable at pH 6.8. Only about 50% of the initial enzymatic activity was lost in 25 days at 0°, even though the protein concentration was 20 μg/ml. A sharp optimal activity for the crystalline deaminase at pH 6.4 (30°C) was demonstrated in 0.1 M succinate buffer. The optimal pH shifted to 6.0 (30°C) when citrate buffer was used. The initial velocity was directly proportional to the enzyme concentration. The Michaelis-Menten constant (Km) was calculated graphically to be 1.43×10^{-3} M. Under the optimal conditions the turnover number is calculated to be 187,000 M/100,000 gm. of protein per minute. Within the experimental error, for every 1.0 mole of adenylic acid (AMP) reacting, 1.0 mole of inosinic acid (IMP) and 1.0 mole of ammonia were formed. No detectable amount of AMP was formed when IMP and NH_3 were incubated under various conditions. Crystalline deaminase did not split the amino group from adenine, 2,6-diaminopurine, adenosine, 2'-adenylic acid, 3'-adenylic acid, ADP, ATP, DPN or TPN in various buffers. 5'-Deoxyadenylic acid was deaminated by crystalline enzyme but the initial velocity was about 1% of that with 5'-adenylic acid as substrate. Orthophosphate, pyrophosphate and fluoride inhibit strongly as described by Nikiforuk and Colowick. Cl^-, Br^-, I^-, CH_3COO^-, NO_3^-, $SO_4^=$, and CF_4COO^- had no effect on the enzymatic activity.

903. EFFECTS OF DIPHTHERIA TOXIN ON MAMMALIAN CELLS CULTIVATED IN VITRO. E. S. Lennox and A. S. Kaplan (intr. by H. H. Mitchell). Depts. of Chemistry and Bacteriology, Univ. of Illinois, Urbana.

With the objective of finding a sensitive assay system for diphtheria toxin to investigate the mechanism of diphtherical toxicity, we have screened several lines of mammalian cells which can be cultivated in vitro. For most of the experiments the criterion of sensitivity was destruction of a monolayer of cells on glass in a suitable medium containing horse or bovine serum. Among the sensitive lines, destroyed by a concentration of approximately 5×10^{-5} γ of toxin nitrogen per milliliter (10^{-4} Lf/ml) are Hela, HEP, rabbit kidney and monkey kidney. "Altered" derivatives (from R. C. Parker, Toronto) of Hela and of monkey kidney are insensitive to a toxin concentration 10^4 times that killing the sensitive parent lines. The growth rate of "altered" Hela cultivated in suspension can be reduced by high toxin concentration (0.1 Lf/ml). Resistance does not seem due to extracellular destruction of toxin. We are comparing metabolism and cell constituents of resistant and sensitive cell lines in the presence and absence of toxin. Results of these studies will be reported.

904. OXIDATIVE PHOSPHORYLATION WITH DIFFERENT MITOCHONDRIAL TYPES. Robert L. Lester and Youssef Hatefi (intr. by L. J. Teply). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

From suspensions of beef heart mitochondria (Crane et al. Biochim. et biophys. acta 22:475, 1956) two kinds of mitochondrial particles (I, II) have now been isolated by differential centrifugation in buffered sucrose. The properties of these particles with respect to oxidative phosphorylation have been characterized. The most easily sedimentible particles (I), which might be thought of as the "native" mitochondrial type, give optimal P/O ratios for glutamate and all the citric acid cycle substrates except isocitrate. The oxidation of exogenous isocitrate with both I and II occurs only upon addition of pyridine nucleotide yielding minimal phosphorylation. The rapid oxygen uptake observed with pyruvate and malate corresponds to complete oxidation to CO_2 and H_2O accompanied by a high P/O ratio. Therefore, the oxidation of enzymatically generated isocitrate is distinct from the cofactor induced oxidation of exogenous isocitrate. The second particle type, which might be considered as a derivative of the original mitochondrion, exhibits much lower rates of oxidation for glutamate and the DPN-linked citric acid cycle substrates. However, the P/O ratios observed for these substrates were only slightly lower than those obtained with I. Oxidation of succinate or isocitrate by II was 2-3 times faster than by I resulting in very low P/O ratios. The transition of I to II can occur upon freezing and thawing. The oxygen-induced aging characteristics of I have been studied and the results confirm the findings of Hunter et al. (Biochim. et biophys. acta 20:237, 1956) insofar as have been examined.

905. REDUCTION OF POSTOPERATIVE NITROGEN DEFICITS BY INTRAVENOUS FEEDING WITH HEXOSE, FAT AND PROTEIN HYDROLYSATES. Stanley Levey, Wm. E. Abbott* and Harvey Krieger.* Dept. of Surgery, Western Reserve Univ. School of Medicine and the Univ. Hosps. of Cleveland, Cleveland, Ohio.

Male patients who had undergone subtotal gastrectomy and were maintained with glucose or fructose solutions during the day of operation and the next 4 postoperative days show a cumulative 5-day nitrogen deficit of approximately 60 gm. Such an intake provides no nitrogen and is deficient in calories and electrolytes. Other male patients who had similar operations and were maintained with a protein hydrolysate containing 10% carbohydrate during a similar period had a 5-day cumulative nitrogen deficit of 20-40 gm. This regimen supplies 12-18 gm of nitrogen, some

sodium, potassium and chloride, but is deficient in calories. A third group of male patients following partial gastrectomy was maintained on a regimen which included the intravenous administration of a fat emulsion in addition to a protein hydrolysate-carbohydrate solution. These individuals showed a 5-day cumulative nitrogen deficit of between 0.5 and 15 gm. This latter regimen provided 0.2-0.4 gm of nitrogen and 25-40 cal/kg/day. These studies demonstrate that the major portion of the nitrogen deficit which occurs following the aforementioned operation is the result of a deficient intake of nutrients rather than the surgical trauma per se. (Supported by grants from the Natl. Insts. of Health, PHS, A-760 (C3), Baxter Labs., Morton Grove, Ill. and the John A. Hartford Fndn.)

906. INTERRELATIONSHIP OF GLUTAMINE AND ASPARAGINE IN PROTEIN BIOSYNTHESIS IN MAMMALIAN CELLS. Leon Levintow. Natl. Insts. of Health, Bethesda, Md.

Several mammalian cells require L-glutamine in the medium for survival and growth in tissue culture (Eagle et al. J. Biol. Chem. 218:607, 1956). One of these strains (HeLa) has been cultivated in a medium containing L-glutamine labeled with C^{14} and with N^{15} in the amide position. Glutamine isolated from the cell protein had the same ratio of C^{14} to N^{15} as the glutamine in the culture medium. There was virtually no synthesis of glutamine from glutamic acid and ammonia under the conditions of the experiments. It therefore appears that the glutamine of the medium is incorporated without prior degradation and is virtually the sole source of the glutamine residues of the cell protein. Glutamine can accordingly be considered an essential amino acid for the nutrition of these cells. The only component of the cell protein, aside from glutamine, which contained appreciable amounts of N^{15} was asparagine. The N^{15} contents of the amide groups of asparagine and glutamine were closely parallel. The implications of this finding with respect to the biosynthesis of asparagine will be discussed.

907. METABOLISM OF ESTRIOL-16-C^{14} IN MAN. Mortimer Levitz, Jean R. Spitzer and Gray H. Twombly (intr. by David K. Fukushima). Dept. of Obstetrics and Gynecology, College of Medicine, New York Univ., New York City.

16-Ketoestradiol-17β-16-C^{14} (J. Biol. Chem. 222: 979, 1956) was treated as sodium amalgam to produce estriol-16-C^{14} (I) (3.3 x 10 cpm/mg) and 16-epi-estriol-16-C^{14} (II). I was separated from II and purified by the following procedure: 1) chromatography on silica gel (methanol-benzene); 2) chromatography on paper (chloroform-formamide) and 3) chromatography again on silica gel. I was distinctly more polar in these systems. It was demonstrated that the maximum contamination of I by II was 0.01%. A human patient received 6.2 x 10^6 cpm of I intramuscularly. The radioactivity excreted the first 3 days were, respectively, 47%, 8% and 3%, of which about 80% was in the glucuronoside fraction. After treatment with β-glucuronidase the extracted metabolites were separated on silica gel and purified further by chromatography on paper. Fifty milligrams each of estriol, 16-ketoestradiol-17β and 16-epiestriol were added to the appropriate fractions. Each was crystallized to constant specific activity and converted to at least 2 derivatives to confirm the radiochemical purity. Itotopic analysis indicated that estriol, 16-ketoestradiol-17β and 16-epiestriol contained, respectively, 62%, 0.9% and 0.3% of the urinary radioactivity.

908. BOUND BIOTIN IN OXALACETIC CARBOXYLASE PREPARATIONS. Herman C. Lichstein. Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.

Further data are presented revealing the presence of biotin in purified preparations of oxalacetic (OAA) carboxylase furnished by Dr. M. F. Utter of Western Reserve University. Previous values for biotin in OAA carboxylase (J. Biol. Chem. 212:217, 1955) were incorrectly given as mμg per gram rather than per mg. Microbiological assays for bound biotin were performed on enzyme samples treated with 3.6 N H_2SO_4 at 121°C for 60 min. Saccharomyces cerevisiae (139) was the assay organism and growth was measured turbidimetrically after 16-20 hr incubation at 30°C. Inhibition of the growth response by homobiotin provided evidence of specificity. The data given reveal the bound biotin content of 5 samples of OAA carboxylase taken from various stages of the purification procedure.

Sample	Sp. Act. OAA Carboxylase	% Diff. Sp. Act.	mμg Bound Biotin/mg Protein	% Diff. Bound Biotin
A	4-6		1.45	
C	15-20	+250	0.75	-48
E	60	+243	1.88	+151
F1,4,5	108	+80	2.83	+51
F2,3,6	47	-56	1.40	-51

Within the limits of error of the methods employed, a significant correlation was found between the degree of purity of enzyme and the concentration of bound biotin. The fall in bound biotin during the first step in purification suggests that the crude material (A) may contain biotin bound to other proteins. (Supported by Office of Naval Research contract NR 135-250.)

909. ACTION OF CARBOXYPEPTIDASE ON ACETYLATED TRYPSIN. I. E. Liener and Robert C. Wong. Dept. of Agricultural Biochemistry, Univ. of Minnesota, St. Paul.

Although trypsin is refractory to attack by carboxypeptidase by virtue of its C-terminal lysine residue, amino acids are readily cleaved from the active, acetylated derivative. The susceptibility of the modified enzyme to the action of carboxypeptidase apparently arises from the fact that vulnerable C-terminal groups are exposed during the self-digestion of acetylated trypsin. Exposure of a 24-hr autolysate of acetylated trypsin to carboxypeptidase at 25°, pH 8, for 96 hr results in the release of at least 35 amino acids from the original molecule. At the same time the system undergoes a marked increase in solubility in 10% trichloracetic acid (TCA), at least 50% of the total nitrogen finally becoming soluble in TCA. These profound changes occur without any detectable loss in enzymatic activity as measured on casein or benzoyl-arginine methyl ester. The digest could be separated into inactive and active components by electrophoresis on a vertical starch column. The active component was found to possess about 50% greater specific activity (on a N basis) than the original enzyme. Results of a preliminary study on the properties of this active degradation product will be presented.

910. MOVING BOUNDARY AND PAPER ELECTROPHORESIS STUDIES ON THE BINDING OF HUMAN SERUM PROTEINS WITH METAL IONS. H. A. Lillevik, H. M. Leduc, D. T. Ewing and P. F. Morse (intr. by J. L. Fairley). Kedzie Chem. Lab., Michigan State Univ., East Lansing, and Harper Hosp., Detroit.

This study was undertaken to determine the extent to which normal human serum albumin and the alpha, beta or gamma globulins are associated with the serum metal ions of calcium, magnesium, iron and copper. Moving boundary electrophoretic analyses for protein distribution and chemical determinations for the metal ion composition were performed on sera of 21 normal adult human beings. Correlation of electrophoretic results with the chemical analytical data indicated that calcium is associated with both albumin and the globulins, magnesium with albumin, iron with beta globulin and copper with gamma globulin. In order to demonstrate more directly the presence of metal ion-protein combinations, the investigation was extended to filter paper electrophoresis of the same sera samples. From each sheet after electrophoresis, a strip was stained with dye to locate proteins and other strips were stained with appropriate reagents to locate metal ions. Calcium was found associated with all of the serum proteins. A strip elution technique revealed that about two-thirds of all serum calcium was distributed equally between albumin (60% of the total serum proteins) and gamma globulin (15% of the total proteins). Magnesium occurred in all serum proteins. Iron appeared only at the beta globulin zone whereas copper and also zinc were associated with gamma globulin. Histochemical stains on paper failed to locate potassium, phosphate, carbonate, chloride and sulfate ions. To determine the effect of buffer, serum and solutions consisting of purified human serum protein with metal ion present was exhaustively dialyzed against 0.1 M veronal of pH 8.6. The extent of metal ion retention by the proteins was determined.

911. SOME OXIDATIVE ENZYMES OF ANAEROBICALLY GROWN YEAST. Aristid Lindenmayer* and Lucile Smith. Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.

A comparison of aerobically and anaerobically grown yeast has led to the identification of several pigments not previously reported. These compounds were observed by means of a specially designed spectrophotometer in intact cells of Saccharomyces cerevisiae (strain LK2G12), grown under strict anaerobiosis; they were not seen in starved aerobically grown cells. The difference spectrum of the anaerobically grown yeast in the presence and absence of oxygen showed peaks at 425, 445, 555, and 585 mμ, which were related to the disappearance of oxygen. A peak at 505 mμ appeared slowly in the difference spectrum, thus was not involved in the immediate changes resulting from the aeration of anaerobic cells. The peak at 505 mμ disappears on addition of sodium dithionate or potassium cyanide in the absence of oxygen. There is also a pigment present which reacts with carbon monoxide to form a compound with an absorption peak at 450 mμ. There is no spectroscopic evidence, however, of the carbon monoxide compound of cytochrome a_3. A third pigment, which has an absorption peak at 420 mμ in the difference spectrum, disappears slowly after the anaerobically grown cells are exposed to oxygen, and does not reappear when the cell suspension becomes anaerobic again. Extracts of the anaerobically grown cells have extremely weak cytochrome c oxidase activity, but very active cytochrome c peroxidase. The extracts can reduce cytochrome c on addition of DPNH or succinate, but not of lactate. Ferricyanide is reduced in the presence of lactate. Possible relationships between the pigments present and the observed enzymatic activities will be discussed.

912. EFFECT OF BOILED ANEMIC PLASMA USED IN CONJUNCTION WITH KNOWN ERYTHROPOIETIC STIMULI. W. H. Linkenheimer and H. Berger (intr. by T. H. Jukes). Research Div., American Cyanamid Co., Pearl River, N. Y.

It is established that the filtrate of boiled plasma obtained from rabbits made anemic with phenylhydrazine will produce a polycythemic response in normal rats. In the present study the filtrate from boiled anemic plasma (BAP) was administered to rats under conditions known to produce erythropoietic stimulation. A group of adult rats was bled an amount equivalent to 2% of body weight. Half of this group was then injected daily with BAP and the other half given saline until recovery of hematocrit values had occurred. Another group was exposed intermittently to a barometric pressure of 310 mm Hg. One-half of these rats received injections of BAP while the other was given saline. A third group was fed cobalt. Some of these animals were injected with BAP and others received saline. Half of an untreated control group received BAP while the others were injected with saline. The injection of BAP produced an increase in the hematocrit values of normal rats, yet did not influence the rate of recovery of bled animals as measured by hematocrit and reticulocyte percentages. The polycythemic response of rats at stimulated altitude was not materially affected by daily BAP injections. However, when BAP injections and cobalt feeding were combined an additive effect in hematocrit values was observed.

913. ALKALINE DEGRADATION OF HYALURONATE OLIGOSACCHARIDES. Alfred Linker, Philip Hoffman* and Karl Meyer. Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.

The action of saturated lime water under nitrogen at room temperature on oligosaccharides obtained enzymatically from hyaluronic acid was studied. N-acetyl hyalobiuronic acid was completely degraded under these conditions to anhydro-acetylglucosamine (Kuhn and Krueger. Chem. Ber. 89:1473, 1956) and glucuronic acid after 6 hr. Glucuronic acid itself seemed to be stable. Tetrasaccharide yielded anhydro-acetylglucosamine and a trisaccharide composed of 2 uronic acids and 1 hexosamine unit. The trisaccharide was not further degraded by the alkali. The other trisaccharide containing 2 hexosamines and 1 uronic acid unit had been prepared enzymatically (Linker, Meyer and Weissmann. J. Biol. Chem. 213:237, 1955). The new trisaccharide was hydrolyzed by β-glucuronidase to glucuronic acid and a disaccharide, having the uronic acid moiety on the reducing end. This repeating unit containing the hexosaminidic linkage of hyaluronate could not be obtained before as hyaluronidases and mineral acids hydrolyze this linkage preferentially. The disaccharide was hydrolyzed by acid or glucosaminidase to its constituent monosaccharides. Oligosaccharides prepared by alkali degradation, having the uronic acid on the reducing end, can be used in biosynthetic and structural studies. They are also being used in elucidating the structure of products obtained by the action of leech enzyme on hyaluronate, which were found to differ from those of testicular and bacterial hyaluronidases, but have similar properties to fractions obtained by the lime water treatment. (This work was supported by grants from the Public Health Service.)

914. PROGRESS ON THE PURIFICATION AND PROPERTIES OF DDT-DEHYDROCHLORINASE. H. Lip

and C. W. Kearns (intr. by R. E. Johnson). Dept. of Entomology, Univ. of Illinois, Urbana.

The purification of DDT-dehydrochlorinase from DDT-resistant houseflies has been continued (Moorefield. Proc. Boyce Thompson Inst. 18:303, 1956). The fractionation of whole fly homogenates instead of acetone powders necessitated an increase in the ammonium sulfate concentration required to affect precipitation of the enzyme. After 10,000-fold purification only half of the enzyme can be precipitated by saturation with ammonium sulfate at various pH. The stability range extends through pH 4-12. Partially purified material is denatured by 1:9 acetone-methanol at -20° and also by Zn^{++} at 2×10^{-4} M. A sharp pH dependance was observed during fractionation with methanol at -15°. When 0.02 M with respect to thiomalic acid, fractionation with methanol at pH 4.1 and 5.9 resulted in a 6-fold purification with 35% yield, whereas at pH 5.0 the purification was 10-fold and the yield 99%. After solvent fractionation, initial solution of the enzyme must be effected in 0.02 M sodium carbonate-versene, pH 12. Following this step, isoelectric precipitation of impurities and sorbtion and elution on calcium phosphate gel resulted in an over-all purification of 50,000-fold with 10% yield.

915. ANTIBODY PRODUCTION TO PHOSPHOHEXOSE ISOMERASE. Marie N. Lipsett,* Ruth B. Reisberg* and Oscar Bodansky. Sloan-Kettering Inst. for Cancer Research, New York City.

Preparations of phosphohexose isomers representing ≥ 40- to 50-fold purification were obtained from perfused dog liver and other tissues by salting-out and adsorption techniques. Rabbits were immunized with purified dog liver isomerase and the antiserum obtained was heat-treated to inactivate the native rabbit serum isomerase. The resulting preparation gave a positive precipitin test down to 2 μg of dog liver isomerase protein. Agar diffusion studies showed at least 8 bands. Tissue specificity was studied for various isomerase preparations. Isomerase from rat liver gave no cross-reaction. Isomerases derived from dog muscle or dog erythrocyte precipitated the antiserum to dog liver isomerase. Dog serum isomerase also reacted with the dog liver antibody. Enzyme recoveries from isomerase mixed with normal rabbit serum were 96-104%. Antiserum suspensions gave complete enzyme recoveries for dog muscle isomerase-antiserum mixtures and no lower than 85% recoveries from dog liver isomerase-antiserum mixtures. Dog liver isomerase was precipitated practically completely after a 4-hr incubation period by the antiserum at ranges between approximately 2 and 20 μg isomerase protein. On either side of this range, the antigen-antibody complex became increasingly soluble. Longer incubation periods, up to 7 days, gave slightly greater amounts of enzyme precipitation in the antibody- and antigen-excess zones but did not appreciably affect the shape of the precipitation curve. The precipitated isomerase-antibody complexes were analyzed for tyrosine content, and ratios for antibody tyrosine to precipitated isomerase tyrosine were calculated for dog liver and dog muscle antigens at different concentrations.

916. ORIGIN OF DEOXYRIBOSE IN THE DNA OF E. COLI. Marilyn R. Loeb* and Seymour S. Cohen. Children's Hosp. of Philadelphia, and Univ. of Pennsylvania School of Medicine, Philadelphia.

Deoxyribose, isolated from E. coli (strain B) grown aerobically on glucose-1-C^{14} as sole carbon source, contained 20-30% of the specific activity of the exogenous glucose. Thus deoxyribose and ribose have comparable activities in this organism (Lanning and Cohen. J. Biol. Chem. 216:413, 1955). In extending this work, the specific activity of the deoxyribose of each nucleoside of DNA was measured to determine whether deoxyribose arises similarly in both purine and pyrimidine derivatives. DNA was isolated via a modified Schmidt-Thanhauser procedure from E. coli grown on glucose-1-C^{14}. The DNA was hydrolyzed by pancreatic DNase and calf intestinal phosphatase to yield the 4 deoxynucleosides in 5-10 micromolar quantities. The deoxynucleosides were separated on Dowex-1-formate and further purified by paper chromatography. The base-labeled and deoxyribose-labeled nucleosides were incubated with a bacterial enzyme and a new unlabeled base to form a new nucleoside in which only deoxyribose was labeled. For example, deoxyribose of C^{14} thymidine was transferred to uracil by this enzyme to form uracil deoxyriboside. The new nucleosides were isolated in high yield (60-80%), characterized, and sugar-derived radioactivity was estimated. The deoxyribose found in each of the nucleosides of DNA had similar activities suggesting a similar mode of origin of purine and pyrimidine deoxyribose from some common precursor. Data from similar experiments of the DNA from $T6r^+$ produced in infected E. coli will also be presented.

917. TRACER STUDIES OF HEXOSE AND PENTOSE FORMATION IN RIPENING FRUIT. Frank A. Loewus and Rosie Jang.* Western Utilization Research Branch, Agricultural Research Service, U. S. Dept. of Agriculture, Albany, Calif.

Ascorbic acid from detached ripening strawberries fed D-glucose-1-C^{14} or -2-C^{14} was found to have 70% of the activity in carbon 1 or 2, respectively (J. Biol. Chem. 222:649, 1956). Significantly greater randomization was found in the ascorbic acid from berries fed D-galactose-1-C^{14}. In order to learn more about the nature of C^{14} randomization in the hexose pool from which ascorbic acid is assumed to be formed, the free sugars from the same berries used above were separated, identified and assayed for radioactivity. Fructose, glucose, sucrose and xylose were present in decreasing order of abundance. However, the specific activity decreased in the order: sucrose, glucose, fructose, xylose for all 3 precursors. Glucose, glucose derived from acid-hydrolyzed sucrose, and xylose were purified chromatographically and degraded microbiologically. In glucose-1-C^{14} fed berries, less than 10% of the C^{14} was randomized in free glucose and between 10 and 15% in sucrose-derived glucose. In galactose-1-C^{14} fed berries, about 15% of the C^{14} was randomized in free glucose and 20% in sucrose-derived glucose. Most of the randomized C^{14} was located in carbon 6. Freee xylose contained a major portion of its activity in carbon 1 when recovered from glucose-1-C^{14} or galactose-1-C^{14} fed berries and in carbon 2 when recovered from glucose-2-C^{14} fed berries. None of the glucoses analyzed contained the high percentage of randomized C^{14} observed in carbon 3 of ascorbic acid. These observations strengthen the view that the initial hexose portion of the oxidative pathway is responsible for ascorbic acid synthesis and that this process occurs parallel with or subsequent to sugar formation in the berry but is probably not intimately connected with the processes responsible for sucrose formation.

918. TRIMETHYLAMINE-YIELDING COMPOUND (TMA-Y) PRODUCED FROM CHOLINE IN RAT LIVER PREPARATIONS. H. B. Lofland,* H. M. Salem* and Camillo Artom. Dept. of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina.

Previous findings on the formation of TMAY during incubation of rat liver mitochondria with methyl- or ethylene-labeled choline (Amer. Chem. Soc., 129th Meeting, Abstracts, p. 10 C) have been confirmed and extended. The compound contains all 5 C of choline, is precipitated by Reinecke acid, and yields trimethylamine (TMA) by alkaline distillation. It was isolated by paper chromatography or ionophoresis, although in the isolated fraction more than 1 molecular species may still be present. TMAY combines with semi-carbazide, but is clearly differentiated from betaine aldehyde by its chromatographic and ionophoretic mobilities, infrared spectrum and lability toward dilute alkalies. Such a lability appears to be enhanced by semicarbazide. In the presence of the latter substance and 0.1 N NaOH at 100° for 1 hr, TMAY yields almost quantitatively TMA (from the methyl-carbons of choline), and glycolaldehyde (from the ethylene carbons). Glycolaldehyde has been definitely identified by paper ionophoresis, formation of hydrazine derivatives and periodate oxidation. Preliminary results of infrared analyses suggest the presence of additional functional groups in TMAY, such as -C-O-C- (ether), and CH_2= CH- (vinyl). Under the conditions of our present and previous experiments, TMAY rather than free TMA is the major, and possibly the only, product of the enzymatic system which we formerly designated as "choline deaminomethylase" to distinguish it from the "choline oxidase" system (3rd Intern. Biochem. Congr., Abstracts, p. 40, 1955). It remains to be determined whether or not, under different conditions, TMAY actually may function as a biological precursor of free TMA. (Aided by Public Health Grant No. A-754 and Atomic Energy Comm. Contract No. AT-(40-1-) 1638.)

919. EFFECT OF GLUCAGON ON GLYCOGENESIS. Robert W. Longley* and Joseph H. Roe. Dorn Lab. for Med. Research, Bradford, Pa. and Dept. of Biochemistry, George Washington Univ., Washington, D. C.

Previous studies in which fasted normal and totally depancreatized rats were used to measure the rate of glycogenesis in the liver, indicated that the maximal response to i.p. injected glucose and fructose solutions occurred around 3 hr after administration. Utilizing the same technique of extracting glycogen by homogenizing the liver in 5% trichloroacetic acid and measuring the glycogen content with anthrone reagent, the effect of glucagon on glycogenesis in the liver has been investigated. Glucose, at a dosage of 2 gm/kg of body weight, was injected i.p. simultaneously with glucagon at dose levels of 1 mg/kg and 5 mg/kg of body weight. In normal animals, there was a marked diminution in glycogen deposition in the liver at 3 hr and 5 hr after injection. When the smaller dose was given, the effect was more marked in the depancreatized rats, less glycogen being deposited, than in the comparable normal animals. In normal animals, despite the fact that glucose was given and very little glycogen was found in the liver, the blood sugar level was in the normal range 3 hr and 5 hr after injection of glucose and glucagon. The depancreatized rats exhibited an elevated blood sugar level, but not of sufficient magnitude to account for all of the glucose injected.

920. pCO_2 CONTROL OF SEXUAL DIFFERENTIATION IN HYDRA. W. F. Loomis. The Loomis Lab., Greenwich, Conn.

Previous results have shown that hydra secrete a volatile compound, or gas, into their culture water that induces them to differentiate sexually whenever the culture conditions are such that this gas accumulates to threshold concentration (Loomis. J. Exp. Zool. 132:555, 1956). This gas has now been identified as carbon dioxide and the differentiation-inducing variable shown to be pCO_2, i.e. the partial pressure of unhydrated CO_2 gas physically dissolved in the medium. A convenient determination of pCO_2 has been devised and it has been shown that pCO_2 is independent of both pH and bicarbonate concentration while it is dependent on such biological variables as population density, depth of culture, degree of stagnation, etc. The extreme lability of the sex-inducing principle has been shown to result from the ease with which dissolved CO_2 gas is lost from solution on even gentle aeration. Since the pCO_2 of aerated cultures is that of air (ca. 0.03% atmosphere), sex-inducing levels of about 0.15% atmosphere can only occur in open cultures that are undisturbed and within which living animals are generating respiratory or other CO_2. The level of pCO_2 present in such cultures depends on the dynamic balance that exists between the rate of CO_2 generation on the one hand and the rate of CO_2 loss from the surface of the culture on the other. Effective means of controlling pCO_2 have been found and pure carbon dioxide gas (Matheson) used to induce sexual differentiation in hydra for the first time. These results indicate that, at least in hydra, pCO_2 constitutes one of the chemical influences active in determining the course of cellular differentiation.

921. A FLUORIMETRIC METHOD FOR THE MICRODETERMINATION OF GLUTAMIC DECARBOXYLASE. Irene P. Lowe, Eli Robins and Geraldine A. Schmalz (intr. by Oliver H. Lowry). Dept. of Psychiatry and Neurology, Washington Univ. School of Medicine, St. Louis.

Because of the uniquely high concentration of glutamic decarboxylase in the central nervous system, the precise histologic localization of this enzyme is of interest. A method has been developed to measure the activity of glutamic decarboxylase in 3 γ, dry weight, of brain. The development of the method depended on the observations that: a) ninhydrin reacts with γ-aminobutyric acid (AB) to yield a highly fluorescent product (measurable at a concentration of 10^{-6} to 10^{-5} M), which has a 10-fold greater fluorescence than the ninhydrin product with glutamic acid (GA). These fluorescent products were formed at 38° in carbonate buffer. Under these conditions and at these dilutions, NH_4 gave no color or fluorescence and there was no visible color with AB or GA. A few other amino acids and serotonin derivatives also gave highly fluorescent products. b) There is an enhancement of the fluorescence of the ninhydrin-AB product when reacted in the presence of GA. c) The fluorescence of the ninhydrin-GA product can be reduced over 90% by the use of 0.03 M Cu^{++} with only a relatively slight reduction of the fluorescence of the ninhydrin-AB product. Measurements of glutamic decarboxylase activity in rat and rabbit brains show activities of 25-35 mM/kg wet weight/hr.

922. 5-AMINO-4-CARBOXY-IMIDAZOLE RIBOTIDE, A NEW INTERMEDIATE IN PURINE BIOSYNTHESIS. Lewis N. Lukens* and John M. Buchanan. Div. of Biochemistry, Massachusetts Inst. of Technology, Cambridge.

The synthesis of 5-amino-4-imidazole-(N-succinylocarboxamide) ribotide (SAICAR) from 5-aminoimidazole ribotide (AIR) by partially purified enzymes from avian liver requires ATP, aspartic acid and bicarbonate. One molecule of ATP is split to ADP and inorganic phosphate for every molecule of SAICAR

which is synthesized. ATP is more effective on a molar basis than GTP, ITP, UTP or CTP. When AIR is incubated with enzyme and bicarbonate in the absence of ATP and aspartic acid, a new compound is formed which has been tentatively identified as 5-amino-4-carboxy-imidazole ribotide (C-AIR). This enzymatic reaction is reversible. When C-AIR is incubated with enzyme, ATP and aspartic acid, it is converted to SAICAR. For this latter reaction bicarbonate is not required, whereas it is required for the conversion of AIR to SAICAR. Proof that the carbon atom of bicarbonate is present in C-AIR was obtained by synthesizing C-AIR enzymatically from AIR and C^{14}-labeled bicarbonate, under which conditions the C-AIR was found to be radioactive. Incubation of this C-AIR with enzyme in the absence of bicarbonate reconverted it to AIR which was non-radioactive. C-AIR is rapidly decarboxylated to AIR by heat or acid. The presence in C-AIR of a primary arylamine group is shown by its reaction in the Bratton Marshall test to give a characteristic dye. The free base, 5-amino-4-imidazole carboxylic acid, was synthesized chemically by the method of Rabinowitz. This compound closely resembles C-AIR in its acid instability and in the spectrum of the dye it produces in the Bratton Marshall reaction.

923. SOME ADSORPTIVE AND CATALYTIC PROPERTIES OF TESTICULAR MICROSOMES. W. Lynn, Rose Brown, and John Mullins (intr. by Frederick Bernheim). Depts. of Biochemistry and Medicine, Duke Univ. School of Medicine, Durham, N. C.

Although microsomes contain no cytochrome c, it has been known for some time that they catalyze the reduction of cytochrome c by DPNH, TPNH and Fe^{++} (Lynn and Brown. Biochim. et biophys. acta 21:403, 1956). We have observed that microsomes have a marked affinity for cytochrome c (but not hemoglobin or myoglobin), Fe^{++} and steroids. Since microsomes are fatty insoluble particles and since they are known to catalyze the oxidation of many insoluble substrates, such as steroids (Slaunwhite and Samuels. J. Biol. Chem. 220:341, 1956) and drugs (Brodie et al. Science 121, 603, 1955), we have begun a survey of their adsorptive properties. By suspending the microsomes in buffer (Tris, pH 7) containing various substrates, resedimenting them (100,000 x g), and assaying for the substrate remaining in the supernatant, we have been able to demonstrate some specificity in their adsorptive abilities, e.g., phosphate is markedly adsorbed, but sulfate not at all; globulins are adsorbed much better than albumins; alcoholic steroids are adsorbed better than ketonic ones; Fe^{++} and Co^{++} are adsorbed but Na, K, Mg are not; fatty acids are adsorbed but amino acids are not; etc. Further, since enzymes, being protein, are adsorbed, there should be some effect of this adsorption on enzyme rates. This has been found to be the case, e.g., alcohol dehydrogenase, lactic dehydrogenase and catalase, in the presence of testicular microsomes, have shown an increased initial rate (50-100%), whereas xanthine oxidase (hypoxanthine as substrate) and hexokinase have shown a decreased rate. Other substances, such as polylysine (Dellert and Strahmann. Nature 176:1028, 1956) and calcium phosphate gel (Keilin and Hartree. Biochem. J. 44:205, 1949), have been shown to increase enzyme rates, but the fact that intracellular "gels" also affect enzyme rates make it appear reasonable that these microsomal surfaces may actually exert some control on intracellular metabolism. Data concerning the effects of various surface active agents on these microsomal effects will also be presented.

924. DIFFERENCE IN GLUCOSE UTILIZATION AMONG INBRED STRAINS OF MICE. John B. Lyon, Jr. (intr. by E. T. Papageorge). Dept. of Biochemistry, Emory Univ., Ga.

The I strain of inbred mice deposits minimal amounts of bodh fat when fed diets containing either 5 or 50% fat. Mice of the C_{57} strain deposit similar amounts of body fat when maintained on the low fat ration but become obese on the high fat ration. Mice of the C_3H and A strains are larger and are more susceptible to nutritional obesity. The fasting blood sugar levels of young adult (3-4 months) mice of all 4 strains fed a stock ration (5% fat) were similar. A dose of either 1 or 3 mg of glucose per gram of body weight elicited similar glucose tolerance curves among the 3 potentially obese strains. At either dosage the tolerance of the I strain was significantly greater although the differences between the 2 groups of mice were not as marked at the lower dosage. These differences in glucose tolerance are ascribable to sharp and sustained rises in muscle (abdominal, gastrocnemius and diaphragm) glycogen of the I strain as opposed to only slight changes in the muscle glycogen concentrations of the other 3 strains. In these 3 strains, most of the administered glucose accumulated in the liver as glycogen. Only small increases in liver glycogen were found in the I strain during the 4-hr interval. When insulin was administered simultaneously with glucose, the increment in muscle glycogen in the I strain (over that seen after glucose alone) was also greater than in the other 3 strains.

925. END-PRODUCT CONTROL FOR THE FORMATION OF A BIOSYNTHETIC ENZYME. Werner Maas and Luigi Gorini.* Dept. of Pharmacology, New York Univ. College of Medicine, New York City.

Although E. coli cells are able to synthesize their amino acids from simple nitrogen and carbon sources, when certain amino acids are supplied in the culture medium the bacteria will utilize them in preference to synthesizing their own. A study of the mechanism underlying this regulation was begun in the case of arginine biosynthesis since most of the steps in this biosynthetic pathway have been characterized in cell-free extracts. It was found that arginine inhibits formation of the transcarbamylase which converts ornithine to citrulline. The inhibition is quite specific: neither citrulline nor ornithine affect the formation of the transcarbamylase. In a growing culture, in the presence of 5 μg/ml of arginine, there is neither increase nor loss of the total enzyme, the preexisting enzyme merely being diluted by cell division. After the removal of arginine from the growth medium the enzyme reappears rapidly. Thus when cells in which the enzyme has been 100-fold diluted, are transferred to medium without arginine, the normal level is restored within half a cell division. The initial rate of enzyme synthesis during the restoration period is 5 times as great as the rate of enzyme synthesis in a culture not preexposed to arginine. Enzyme restoration requires the presence of all amino acids: it does not take place in an amino acid auxotroph, in the absence of its required amino acid. These findings suggest end-product control for enzyme appearance at the level of de novo protein synthesis.

926. CHLORPROMAZINE AND AZACYCLONOL AND THE IN VITRO LABELING OF PHOSPHATIDES IN BRAIN PREPARATIONS. W. L. Magee, E. T. Pritchard and K. P. Strickland (intr. by R. J. Rossiter). Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.

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It was previously shown (Biochim.et biophys. acta 21:408, 1956) that 2 drugs with described tranquillizing properties, chlorpromazine and azacyclonol, cause an increase in the labeling of lipid P from inorganic P^{32} in respiring slices of guinea pig brain, with no change in the oxygen consumption or rate of glycolysis. There is also no change in the labeling of ATP, or in the concentrations of ATP and creatine phosphate. The effect is obtained with concentrations of the drugs considerably lower than those necessary to inhibit oxygen consumption and aerobic phosphorylation. It is now reported that a similar effect, although smaller, is obtained in mitochondrial preparations from rat brain and that the effect is almost absent from glycolyzing homogenates. With guinea pig brain slices and inorganic P^{32} as the source of radioactivity, the increased labeling is confined to diphosphoinositide, phosphatidic acid and phosphatidyl serine. The labeling of phosphatidyl choline (lecithin) and phosphatidyl ethanolamine is decreased. The increase in labeling is also observed when the radioactivity is derived from glycerol-1-C^{14} or serine-3-C^{14}, but with choline-1,2-C^{14} as the source of the radioactivity, the labeling of phosphatide is decreased. With acetate-1-C^{14}, there is no significant change.

927. METABOLISM OF 7-KETOLITHOCHOLIC ACID-24-C^{14} IN THE RAT. Theodore A. Mahowald,* M. W. Yin* and Edward A. Doisy. Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.

Radioactive 7-ketolithocholic acid was prepared by oxidation of chenodeoxycholic acid-24-C^{14} with chromic acid at ice bath temperature. The sodium salt of 1 mg of the labeled 7-ketolithocholic acid (3.09×10^6 d/m/mg) was administered by stomach tube to rats with bile fistulas and the C^{14} determined in the excreta. Nearly all of the carbon 14 was recovered in the bile during the first 72 hr. The bile from 1 animal was hydrolyzed with 5% NaOH in the autoclave at 15 lb for 3 1/2 hr. The free bile acids were extracted and chromatographed on an aqueous acetic acid partition column. Of the chromatographed radioactivity, 71% appeared in the dihydroxycholanic acid zone. On the basis of chromatographic behavior and isotopic dilution, more than half of this C^{14} appeared to be in ursodeoxycholic ($3\alpha, 7\beta$-dihydroxycholanic) acid. Another 15% of the C^{14} which was present in the acid I zone was identified as acid I by isotopic dilution experiments. A small fraction amounting to only 3.5% of the C^{14} was found in the acid II zone. In comparison with these results, after administration of chenodeoxycholic acid-24-C^{14}, 15% of the acid was metabolized to acid I and 20% to acid II and no radioactive ursodeoxycholic acid could be detected (Mahowald et al. J. Biol. Chem., in press).

928. INCORPORATION OF P^{32}-ORTHOPHOSPHATE INTO THE PHOSPHATIDES OF ISOLATED RAT LIVER MITOCHONDRIA. G. V. Marinetti, J. Erbland* and M. Albrecht.* Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The incorporation of P^{32}-orthophosphate into the lipides of intact rat liver mitochondria occurs mainly into phosphatides which resemble phosphatidic acids and which constitute only 7% of the total lipide P. The incorporation into the common phosphatides such as lecithin and phosphatidyl ethanolamine is extremely small. Glycerol, succinate, magnesium, ATP and CTP were found to stimulate the incorporation into the phosphatidic acid-like lipides and into 2 other unidentified phosphatides. The microsomal-rich supernatant fluid also had a stimulating effect. On the other hand, cyanide, dinitrophenol, malonate, calcium fluoride and a variety of metal ions and surface agents were found to inhibit the system. The maximum amount of incorporation occurred after 1 hr incubation at 37°. The addition of stearic acid, ceramide, phosphatidic acid, choline, serine and ethanolamine had little effect on the system. The identification of the mitochondrial lipides was made by chemical, infrared spectral and paper and column chromatographic analysis. Lecithin and phosphatidyl ethanolamine were found to be the predominant phosphatides of mitochondria. Incubation of the mitochondria under the condition of these experiments gave rise to appreciable amounts of lysolecithin. In contrast to intact mitochondria, acetone powders of these particles were far less effective in incorporating P^{32}-orthophosphate into the phosphatides. However, a striking difference was found inasmuch as in these latter systems the incorporation occurred solely in lecithin and phosphatidyl ethanolamine and none occurred in the phosphatidic acid-like lipides.

929. DETECTION OF SMALL QUANTITIES OF BOUND AMINO ACIDS IN THE PRESENCE OF LARGE QUANTITIES OF FREE AMINO ACIDS. Alvin Markovitz (intr. by Carl Baker). Lab. of Metabolism, Natl. Heart Inst., Bethesda, Md.

A method for measuring 1 part peptide in the presence of 100 parts free amino acid has been developed. Free amino acids, but not peptides other than N-terminal glycine peptides, are destroyed by reaction with ninhydrin. The ninhydrin is then destroyed with hydrogen peroxide. The residue is then hydrolyzed with acid and the amino acids determined in the usual way. Results with glycyl-L-leucine show that the N-terminal amino acid is destroyed; thus recovery of carbon dioxide from such a peptide amounts to 55% of theoretical. Other synthetic di- and tripeptides are recovered in quantities of from 68 to 93% depending on the peptide employed. Studies on the destruction of free amino acid have shown that at least 99.7% of the free amino acid is destroyed by ninhydrin under the conditions employed. Thus, any unknown sample revealing more than 0.3% peptide indicates the presence of bound amino acids (peptides?). The method is particularly useful as an extremely sensitive device for the detection of radioactive peptides in the presence of large excesses of radioactive free amino acids, even when the net amounts of peptide material are very low. Applications of the method described will be presented.

930. OXIDATIVE PHOSPHORYLATION DURING MATURATION OF PLANT TISSUE. Joy D. Marks and J. E. Varner (intr. by M. L. Wolfrom). Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

Oxygen consumption markedly increases during the ripening of many fruits, resulting in a "climacteric maximum" of respiration, followed by a postclimacteric respiratory decline. Robertson and Turner (Australian J. Sci. Research Series B4:75, 1951) proposed that the occurrence of limited respiration during the preclimacteric period of fruit development may be due to the limited availability of phosphate acceptors. Oxidative phosphorylation in intact tomato fruit has been investigated by P^{32} tracer techniques and shown to be operative during early (preclimacteric) ripening and likewise, during the postclimacteric period of senescence. The percentage of P^{32} incorporated into organic esters by oxidative phosphorylation reached a maximum in early, preclimacteric fruit, and did not decrease until after the onset of pink color. It was

possible to prevent phosphorylation by the uncoupling action of dinitro phenol and also by bruising injury, but complete disappearance of phosphorylating capacity did not occur naturally until the termination of senescence. Green mature fruit failed to ripen normally when treated with dinitrophenol, or after bruising. The role of oxidative phosphorylation in fruit ripening would thus point toward the existence of energy requiring syntheses, which are essential to the completion of ripening by senescing fruit. That climacteric is induced by increased availability of ADP would similarly be consistent with the concept of fruit ripening as a process initiated by synthesizing activities.

931. DEMONSTRATION OF NET PLASMA ALBUMIN SYNTHESIS IN LIVER SLICES FROM NORMAL AND NEPHROTIC RATS. Julian B. Marsh and David L. Drabkin. Dept. of Biochemistry, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

Previous labeling studies from this laboratory (Drabkin and Marsh. J. Biol. Chem. 212:623, 1955) indicated an increased synthesis of plasma albumin by the nephrotic rat. A direct demonstration of such increased synthesis has now been achieved in vitro with rat liver slices, in a manner similar to the chicken liver slice experiments of Peters and Anfinsen (J. Biol. Chem. 186:805, 1950). A net synthesis of plasma albumin by the rat liver slices occurred in a medium of the following composition (in mM/liter): KCl, 77; NaCl, 39; $NaHCO_3$, 32.5; $MgSO_4$, 3.1; $CaCl_2$, 1.25; KH_2PO_4, 0.6. The gas phase was O_2, 95% - CO_2, 5% and final pH of 7.3. No substrates were added to the medium. The slices were washed in the medium for 45 min at 37° and then transferred to fresh medium for an additional 2 hr of incubation. Before and after incubation, slices and medium were homogenized and aliquots of the supernatant fluid were analyzed for albumin content by means of a quantitative precipitin reaction with rabbit anti-serum, prepared against purified rat plasma albumin. With this technique, the net synthesis of plasma albumin by liver slices from 7 normal rats (controls) averaged 322 ± 26 γ per gm wet weight per 2 hr. The corresponding value for liver slices from 9 nephrotic rats was 584 ± 40 γ, or an increase of 81%.

932. CHARACTERIZATION OF ORGANIC ACIDS BY MICRODIFFUSION. Lawrence M. Marshall. Robert A. Taft Sanitary Engineering Ctr., Cincinnati, Ohio.

During partition studies of the chemical nature of carbon compounds in urban air, a procedure was devised for the identification of volatile organic acids in microgram quantities. The procedure is based on the principles underlying the characterization of such acids through the use of Duclaux constants. Microdiffusion cells are employed and the organic acid in aqueous solution is placed in the outer vessel of the cell. Sodium hydroxide pellets (1 or 2) in the inner well provide the force for the microdistillation. The percentage rate of acid transfer to the inner well was related to the molecular weight of the acid under controlled conditions. In a typical experiment, when the volume of an 0.2% solution of organic acid was 0.2 ml, the capacity of the outer vessel of the Conway cell 2 ml and the temperature 23°, 11, 14 and 27% of acetic, butyric and hexanoic acids, respectively, escaped from the outer vessel of the cell in 2 hr. It was found that the percentage rate of escape of the organic acid in 0.2% solution did not differ greatly from that in 2% solution. The volume of organic acid solution, as would be expected, was 1 factor found to determine the quantity of acid that would enter the center well in any fixed time period. Rate of escape of the acid increased with increase in temperature. In the absence of rigid temperature control, the microdiffusion of an unknown acid must be measured simultaneously with a (any) known volatile acid and this is the single observed limitation to date.

933. PURIFICATION AND INTERACTION OF PROTEINASE C WITH A SKIN INHIBITOR. Charles J. Martin (intr. by A. E. Axelrod). Biochemistry Dept., Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The reversible inactivation of proteinase activity in rat skin extracts induced by changes in ionic strength has been an object of study in this laboratory (Martin. Fed. Proc. 13:1954; Martin and Axelrod. J. Biol. Chem., in press). The time and temperature dependent, pH independent inactivation proceeded maximally upon exposure of the extract to ionic strength 0.6-0.8 and was postulated to occur by association with an inhibitor (CIn) also present. The activation or dissociation reaction occurred instantly upon exposure to ionic strength 1.6. The participating enzyme, designated proteinase C, has now been purified by means of dialysis, alumina gel adsorption and methanol precipitation to a state wherein its activity is refractory to ionic strength activation or inactivation conditions, i.e., the CIn inhinitor has been removed. It will also be shown that a CIn preparation, free of proteolytic activity, has been obtained. The results of studies concerned with the purified proteinase C-CIn interaction has confirmed the postulate that, an initial rat skin extracts, the reversible inactivation of proteinase C occurs by association with an inhibitor. Some properties of the proteinase C preparation, in particular its dependence upon salt concentration for the optimal rate of casein digestion, will also be discussed.

934. ABSORPTION OF RADIOACTIVE IODINE LABELED SERUM ALBUMIN FROM THE KNEE JOINT SPACE IN TREATED AND UNTREATED ARTHRITIS. W. E. Martindale, W. L. Hawley and H. L. Holley (intr. by W. J. Wingo). Radioisotope Service, VA Hosp., and Dept. of Medicine, Univ. of Alabama Med. College, Birmingham.

Ten patients with rheumatoid arthritis were injected intraarticularly (affected knee joint) with I^{131}-labeled human serum albumin (IHSA). Administration of Lugol's solution, which was begun 1 day before injection, was continued throughout the experiment. An aliquot of IHSA containing activity equal to that injected (9.2 μc) was reserved for later use as a counting standard. Serial blood samples were drawn and counted in a scintillation well counter. At the end of each experiment (at least 48 hr after injection) the blood volume was determined separately. From 1) the activity injected, 2) the activity in serial blood samples and 3) the total circulating blood volume, the tagged protein in the circulation was estimated. In untreated subjects, from 9% to 18% of the injected material was in the circulation at the 4th hr. In patients undergoing parenteral steroid therapy less than 4% of the labeled protein was in the circulating blood at the 4th hr. These data, together with counts taken at other intervals, suggest an increased rate of passage of the labeled protein from the knee joint space in the untreated arthritic.

935. EFFECTS OF L-THYROXINE ON YEAST RESPIRATION. W. Marx and M. Gutenstein.*

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Resting cell suspensions were prepared a) of Fleischmann's "Active Dry Yeast" and b) of a pure yeast strain, Saccharomyces cerevisiae. The cells of the former were suspended in phosphate buffer, pH 7.0, those of S. cerevisiae in Tris buffer, pH 7.4. The effects of L-thyroxine on the respiration of these resting cell suspensions were studied using conventional manometric techniques. It was observed that the hormone, at concentrations of 10^{-2} to 10^{-13} M, increased the Q_{O_2} of cells of Fleischmann's yeast by about 30 - 70%; the Q_{O_2} of S. cerevisiae suspensions was also significantly stimulated by L-thyroxine concentrations of 10^{-6} to 10^{-10} M.

936. CHEMICAL NATURE AND CATALYTIC FUNCTIONS OF THE IRON MOIETIES OF SUCCINIC DEHYDROGENASE. V. Massey. Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.

Previous studies in this laboratory have shown that acid or thermal denaturation of succinic dehydrogenase liberates the entire iron content of the enzyme (4 atoms/mole) as inorganic iron, all of which appears in the ferrous state in the o-phenanthroline method. It has been found that when liberated in the presence of sulfhydryl binding reagents, such as p-chloromercuriphenyl sulfonate, the iron is almost entirely in the ferric form. The reaction of the native enzyme with mercurials results in extensive bleaching, particularly in the spectral region where the contribution of iron-protein bonds is maximal. This spectral change is accompanied by a loss of iron from the enzyme. It appears then that the iron of the purified dehydrogenase is largely in the oxidized form, and that its appearance as reduced iron in the o-phenanthroline method is due to secondary reduction by -SH groups liberated on denaturation. The differential effects of chelation of the iron moieties of succinic dehydrogenase on the reactions of the enzyme with various electron carriers have been studied and the results will be discussed in terms of possible sites of action and reaction mechanisms of these carriers with the dehydrogenase..

937. MACROMOLECULAR PROPERTIES OF ISOMERIC CHONDROITIN SULFATES. Martin B. Mathews. Dept. of Pediatrics and Biochemistry, Univ. of Chicago and LaRabida Jackson Park Sanitarium, Chicago, Ill.

Three different acid mucopolysaccharides from connective tissue have disaccharide repeated units containing equimolar amounts of uronic acid, hexosamine, N-acetyl and ester sulfate. Physical-chemical data were obtained in order to compare the macromolecular structures of the sodium salts of these substances. Chondroitin sulfate B (CH-B, β-heparin from beef lung, courtesy of A. Winterstein) was fractionated with alcohol. Fraction 3 had $[\eta]$ = 0.57, M_N = 15,500, M_W = 22,000; fraction 6 had $[\eta]$ = 0.34, M_N = 10,000. These values indicate a relationship between $[\eta]$ and M similar to that for chondroitin sulfate A (CH-A from beef cartilage, Arch. Biochem. 61:367, 1956). The viscosity functions in water of CH-B fit the Fuoss equation for linear polyelectrolytes with constants comparable to those for CH-A of the same M (Arch. Biochem. 43:181, 1953). Potentiometric titrations, however, show the intrinsic pK_o of the carboxyl group to be 0.5 pH unit higher in CH-B than in CH-A. Chondroitin sulfate C (CH-C from chordoma, courtesy of K. Meyer) had $[\eta]$ = 0.84, M_N = 35,000. These constants are near values for undegraded CH-A. Viscosity functions in water are likewise similar for CH-C and CH-A. Thus, these mucopolysaccharides are linear polyelectrolytes with similar chain configuration-charge relationships. Implications for fine structure of minor differences in physical properties will be discussed. (Supported by a grant from the Chicago Heart Assoc.)

938. METABOLITES OF HYODEOXYCHOLIC ACID IN SURGICALLY JAUNDICED RATS. J. T. Matschiner,* R. L. Ratliff* and E. A. Doisy, Jr. Depts. of Biochemistry and Internal Medicine, St. Louis Univ. School of Medicine, St. Louis, Mo.

Previous studies in this laboratory have demonstrated quantitative changes in the metabolism of several labeled bile acids in rats under conditions of bile duct ligation. A marked example of such changes was observed after the administration of hyodeoxycholic acid-24-C^{14} to rats with bile fistulas or with ligated bile ducts. Whereas 94% of the administered C^{14} was recovered in the bile as hyodeoxycholic acid, less than 7% of the C^{14} was recovered in the urine unchanged (Matschiner et al. Fed. Proc. 14:252, 1955). Further studies on the urine of surgically jaundiced rats after the intragastric administration of 40 mg/day of hyodeoxycholic acid-24-C^{14} have led to the isolation of 2 metabolites, acid I and another acid (m. 196°; $[\alpha]_D^{26}$ = +33°) chromatographically similar to hyocholic acid. This new acid reacts with periodic acid indicating a glycol structure but it is not identical with any of the 3 previously reported 3α, 6, 7-trihydroxycholanic acids.

939. ISOLATION OF A CRYSTALLINE ENZYME THAT CLEAVES HOMOSERINE AND CYSTATHIONINE. Yoshihiko Matsuo* and David M. Greenberg. Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

A yellow protein has been isolated in the crystalline state from rat liver which catalyzes both the deamination of homoserine to α-ketobutyrate and the cleavage of cystathionine to α-ketobutyrate, ammonia and cysteine. The assay method employed in the purification procedure was the formation of α-ketobutyrate from homoserine. The method of isolation involved controlled heat denaturation, fractionation with ammonium sulfate, alcohol precipitation and treatment with protamine sulfate. Crystallization is obtained from a 45% saturated ammonium sulfate solution at about 0°, due to the negative temperature coefficient of solubility of the protein. The purified enzyme has absorption maxima at 280 and 427 mμ, measured in 0.2 M phosphate buffer, pH 7.5. The yellow color is not removed upon prolonged dialysis against this buffer. Addition of cyanide, cysteine or bisulfite, however, causes the immediate disappearance of the color. This suggests that the yellow color is due to the presence of pyridoxal phosphate, which has been reported to be the coenzyme of both homoserine deaminase and cystathionase. The ratio of activities on the 2 substrates has remained constant during purification and preliminary efforts to separate the 2 enzyme activities including attempts at differential heat denaturation have been unsuccessful. The rate of formation of α-ketobutyrate from DL-homoserine and from DL-(and allo-)cystathionine is of approximately equal magnitude. The optimum pH for both substrates is the same, namely pH 8.3, upon incubation for 20 min at 37°.

940. LIPOPROTEIN COMPLEX, NUCLEIC ACIDS, AND OTHER CONSTITUENTS OF BOVINE SPERMATOZOA. Dennis T. Mayer, Frank Madera-Orsini* and Robert E. Berry.* Dept. of Agricultural Chemistry, Univ. of Missouri, Columbia.

Basic concepts of spermatozoan chemistry are an outgrowth of studies by Miescher and Kossel on fish spermatozoa and the literature contains a paucity of reports on the individual molecular moities contributing to the structure of these cells. The present investigation with bovine spermatozoa is a continuation of previous work in this laboratory on boar spermatozoa. A fraction soluble in 0.01 N NaOH solution and precipitable with acetic acid (pH 6) was isolated from liophilized bovine spermatozoa. This fraction, after purification, was found to be a lipoprotein similar to that isolated from rat liver nuclei and boar spermatozoa. It constituted about 42% of the dry weight of bovine spermatozoa. Qualitative and quantitative studies showed that the lipoprotein is a complex containing phospholipid, protein, cholesterol, glucosamine and glucuronic acid. Analyses showed 13.8% nitrogen, 0.81% phosphorus and 30% lipid materials. The protein of the complex contained 14.6% nitrogen and 0.5% phosphorus. The residue, after removing the lipoprotein, contained a keratin-like fraction which was removed by extraction with thioglycollic acid and subsequent neutralization with NaOH solution. From the final residue nucleic acid (DNA), about 7% of the dried residue, was isolated by deproteinization with chloroform-octanol mixture. Tests indicate incomplete removal of DNA and the presence of DNA degradation products in the final residue.

941. CAROTENE METABOLISM IN ESSENTIAL FATTY ACID DEFICIENCY. J. S. McAnally and Chester Szymanski (intr. by George T. Lewis). School of Medicine, Univ. of Miami, Coral Gables, Fla.

In an earlier investigation it was noted that the utilization of carotene in the essential fatty acid-deficient rat was affected by the inclusion of small quantities of a linoleate ester in a "fat-free" diet. It was also observed that the ultraviolet absorption spectra of the nonsaponifiable fraction of the liver was different in rats supplemented with vitamin A from the spectra obtained from the same fraction from rats supplemented with 10% α- 90% β- carotene. In the present study, rats were maintained on a diet deficient in essential fatty acids but containing 3% fat. After the deficiency symptoms had appeared, the animals were supplemented variously with vitamin A, β-carotene, α-carotene, and 10% α- 90% β-carotene; in each case, both with and without added linoleate. The effect of each of the provitamins on the absorption spectrum of the nonsaponifiable liver fraction was determined for the 300-350 mμ range as compared with the spectrum obtained after administration of vitamin A. Additionally, the essential fatty acid content of the small intestines were determined and the values obtained were compared with efficiencies of carotene utilization. The over-all efficiencies of carotene utilization, as measured by vitamin A storage in the liver, have been calculated and the effect of linoleate supplementation has been measured.

942. DESCRIPTION OF Se-75 DISAPPEARANCE FROM DOG BLOOD. Kenneth P. McConnell and Dorothy M. Roth.* Radioisotope Service, VA Hosp., and Univ. of Louisville Med. School, Louisville, Ky.

Experiments were carried out to study the rates of change of Se-75 content in the various components of blood. To accomplish this a 20-kg dog was injected subcutaneously with 1.4 mc $Se^{75}Cl_4$ dissolved in 0.61 N HCl, representing 2.14 mg Se. At frequent intervals up to 310 days samples of whole blood, plasma and washed red blood cells were obtained and Se-75 activities were recorded and plotted against time. The curves were assumed to be of the form $Y = ae^{-kx}$, and the values for the a and k parameters were calculated by the method of least squares and half-time values were determined. The r ate of disappearance of Se-75 from whole blood can be described as a single exponential function, while that for plasma is a 2 component rate function. The disappearance of Se-75 from the red blood cells appears to be a multiple-component rate function with the greatest rate of disappearance occurring during the 91st and 126th days after injection of Se-75.

943. EFFECT OF TEMPERATURE ON VELOCITIES OF ELECTROMIGRATION AND ON ELECTRO-OSMOSIS. Hugh J. McDonald and Edward W. Bermes, Jr.* Dept. of Biochemistry, Grad. School and Stritch School of Medicine, Loyola Univ., Chicago, Ill.

The effect of temperature on velocity of electromigration through paper stabilized electrolytes, for a range of substances varying from low to high molecular weight materials, was investigated. The substances studied included: bromphenol blue, bovine plasma albumin, human serum albumin and globulin fractions, and human serum lipoproteins. The ionographic apparatus employed utilized a horizontal cellulose or glass paper strip in a water-saturated atmosphere. The runs were made at temperatures ranging from 4 to 25°C, and under constant conditions of veronal buffer, ionic strength, pH and potential gradient. The paper strips were permitted to stand, after wetting and with the current on, to reach a constant value for the ratio of "weight of buffer solution" to "weight of paper" before beginning a run. The velocity of electromigration of all materials studied in electrolytes stabilized with cellulose paper increased logarithmically with increase in temperature of the buffer solution and the effect of temperatures on different migrants was nearly uniform. To determine the effect of temperature on electro-osmosis, the movement of dextran on glass paper was determined, since it is known that electroosmosis is very much greater on glass paper than on cellulose paper (Biochim et biophys. acta 20:416, 1956). It was found that the effect of temperature on electroosmotic movement, as measured by dextran, also increased logarithmically with rise in temperature. Both electromigration rate and electroosmotic velocity can be represented by the equation: $\log(\text{mobility}) = m \cdot 1/T + B$, where T is °Kelvin, B is a constant and m is the slope of the line obtained when the logarithm of the mobility is plotted against the reciprocal of the absolute temperature.

944. EXTRA-PANCREATIC, EXTRA-SALIVARY AMYLASE IN THE RAT. Robert L. McGeachin and John R. Gleason (intr. by John Fuller Taylor). Dept. of Biochemistry, Univ. of Louisville School of Medicine, Louisville, Ky.

Neither pancreatectomy (Roe, Smith and Treadwell) nor salivarectomy alone in rats affects serum amylase levels. We have now performed the double operation on rats using the technique of Treadwell and Roe for the pancreatectomies. These rats had normal serum amylase levels although their blood glucose levels were markedly elevated and analyses indicated at least 98% removal of their pancreases. Amylase levels were determined by the amyloclastic method of Van Loon whose units are numerically equal to Somogyi's.

Serum amylases in 3 rats surviving both operations were 2580, 3130 and 2790 compared to an average of 2730 in 14 normals. Their mouths were dry and saline washings of the mouths contained no amylase whereas washings from normal rats were powerfully amylolytic. Amylase levels of the liver, kidney, spleen and lung were not significantly different from the normal but the amylase levels of the duodenum and colon were lower suggesting that much of the normal duodenal amylase is pancreatic in origin. Since removal of both known sources of amylase in the rat does not affect serum and tissue amylase levels, previous suggestions (Somogyi; Roe, Smith and Treadwell; Wiberg and Tuba) that there must be an extra-pancreatic, extra-salivary site of amylase synthesis in the rat are conclusively confirmed. (Supported by grant C-2601 C from the Natl. Cancer Inst., Natl. Insts. of Health.)

945. INACTIVATION OF INFECTIOUS NUCLEIC ACID FROM TOBACCO MOSAIC VIRUS BY ULTRAVIOLET LIGHT (2537Å). A. Douglas McLaren and William N. Takahashi.* Soils and Plant Nutrition and Plant Pathology Depts., Univ. of California, Berkeley.

The quantum yield for the inactivation of tobacco mosaic virus (TMV) is very low as compared with typical proteins. Considering the size of the virus, however, it is remarkable that the yield is as high as it is (McLaren, Gentile, Kirk and Levin. 1953) and it has been suggested that the nucleic acid in the virus may have a greater sensitivity to UV than would a biochemically active protein molecule of the same size (Bawden and Kleczkowski. 1955). We have found that infectious nucleic acid (RNA) from common TMV (Schuster, Schramm and Zillig. 1956) is about 6 times as sensitive to inactivation by UV as it is in the form of intact virus. Inactivation takes place, by a 1-hit process at pH 7.3 in 0.02 M phosphate buffer, without a reduction in intrinsic viscosity (210 - 280) at 6°, but with a slight reduction in optical density at 2600Å (less than 1%). It is clear that inactivation involves only a local change on the RNA chain. The energies required for 50% inactivation of the following substances in joules per milligram are: nucleic acid (free), 0.43; nucleic acid (in the intact TMV, calculated), 2.7; TMV, 0.15; enzymes and antibodies, 0.75. (Supported in part by the Atomic Energy Comm.)

946. PENTOSE METABOLISM IN HUMAN ERYTHROCYTE GHOSTS. William L. McLellan and Fabian J. Lionetti (intr. by Burnham S. Walker). Dept. of Biochemistry, Boston Univ. School of Medicine, Boston, Mass.

The pentoses of nucleosides have been found to be extensively metabolized in erythrocyte ghosts. Column chromatographic separations (Dowex #1 Cl) of trichloroacetate extracts of ghost suspensions equilibrated for 1/2, 1 1/2 and 3 hr with inosine at 37C yielded a buildup and a turnover of ribose-5-phosphate and ribulose-5-phosphate. Subsequently, these esters were metabolized to fructose-6-phosphate and fructose 1,6-diphosphate. The pentose-phosphates and fructose-6-phosphate reached a maximum in 1 1/2 hr followed by decreases. The fructose 1,6 diphosphate and glucose-6-phosphate increased to 3 hr. There was no detectable sedoheptulose-7-phosphate which may be rapidly transient in the system. Monophosphoglyceric acid and 2,3 diphosphoglyceric acids increased for 3 hr. No detectable phosphoglyceraldehyde (as alkali labile phosphorus) was detected. Low levels of adenine nucleotides initially present decreased significantly between 1 1/2 and 3 hr. The time variation of specific activities of phosphate esters from equilibrations of ghosts suspensions with P-32 as phosphate and inosine, are consistent with the phosphorolytic cleavage of inosine to form ribose phosphates. Ribose-5-phosphate is converted to fructose-6-phosphate and this in time becomes glucose-6-phosphate. The specific activity of phosphoglyceric acid was appreciably higher than fructose 1,6-diphosphate in 1/2 hr, indicating the production of trioses from reactions other than aldolase. Large changes in the specific activities of adenine nucleotides occurred including an unexplained increase in adenylic acid. The erythrocyte ghost therefore, as a tissue incapable of glycolysis (no glucose utilization) shows the erythrocyte to contain the major components of an anaerobic hexose monophosphate shunt.

947. EFFECT OF PURINE RIBOSIDES ON GLUCOSE AND PYRUVATE METABOLISM OF HUMAN ERYTHROCYTES. Thomas J. McManus, Searle B. Rees and John G. Gibson, 2nd. (intr. by Harry C. Trimble). Depts. of Medicine, Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.

We have shown that intact erythrocytes and ghosts can metabolize the pentose moiety of purine ribosides (adenosine, inosine, guanosine) resulting in a net accumulation of acid-soluble ester phosphate and lactate (Lionetti, et al. J. Biol. Chem. 220:467, 1956). Other investigators have observed either no effect or a stimulus to net glucose disappearance. In the presence of adequate concentration of inorganic phosphate we find an apparent inhibition of glucose consumption with unimpaired lactate production. The interference of pentose reducing substance is ruled out by the use of a specific enzymatic method for estimating glucose. In systems free of glucose, purine riboside yields significant lactate production and does not cause the appearance of nonphosphorylated reducing substance. When glucose consumption is stimulated by the addition of methylene blue, purine riboside is still able to effect significant inhibition. However, studies conducted in the Warburg apparatus demonstrate that in the presence of methylene blue these compounds increase the rate of oxygen consumption over that seen with glucose alone. Purine ribosides plus glucose are additive and result in oxygen uptake more than 2 times greater than that seen in the glucose controls. Pyruvate is also a significant co-factor in the metabolism of purine ribosides by erythrocytes. Fortification of the system with pyruvate in amounts comparable to the glucose and purine ribosides present results in a net disappearance of pyruvate with the appearance of stoichiometric amounts of lactate. The controls show minimal amounts of pyruvate utilized.

948. AN EPSILON LYSYL PEPTIDE FROM COLLAGEN. Gerald L. Mechanic* and Milton Levy. Depts. of Biochemistry, Colleges of Medicine and Dentistry, New York Univ., New York City.

Achilles tendon collagen from cattle was partially hydrolyzed in 7 N HCl at 23-4° for 48 hr. Chromatographic examination of the hydrolysate on Dowex 50 by elution with 0.2 M ammonium formate gave a ninhydrin peak emerging at pH 3.38. This peak contained 2 chromogenic materials which were separated on paper chromatograms using butanol saturated with 0.05 M trichloroacetic acid. One of the materials is a tripeptide containing glycine, lysine and glutamic acid. Amino end group determination with pipsyl-chloride showed glycine to be N terminal. Traces of pipsyl glutamic acid were also found. Treatment of the tripeptide with nitrous acid followed by acid

hydrolysis yielded 2 hydroxy 6 amino hexanoic acid identified by comparison with a synthetic product obtained by nitrous acid treatment of epsilon benzoyl lysine. The treatment used deaminates N terminal glycine and the adjacent amino group in glycyl peptides. The ratio of ninhydrin color values by the Troll and Cannan method before and after hydrolysis of the tripeptide is 1 : 1.5. It has been observed that, while lysine gives color nearly equal to a mono amino acid in this method, epsilon lysyl groups in peptide combination give 1/2 the color of free lysine. The peptide is therefore believed to be either alpha glycyl epsilon (alpha glutamyl) lysine or epsilon (glycyl-glutamyl) lysine. Synthesis of these compounds is in progress.

949. STUDIES WITH CARBOXYL- AND RING-LABELED 3-HYDROXYANTHRANILIC ACID. Alan H. Mehler and E. L. May.* Natl. Insts. of Health, Bethesda, Md.

An established route of tryptophan metabolism involves oxidation through kynurenine and 3-hydroxykynurenine and formation of 3-hydroxyanthranilic acid, which is a precursor of nicotinic, quinolinic and picolinic acids. Since it has been shown that one of the enzymes involved in 3-hydroxyanthranilic acid metabolism, picolinic carboxylase, changes in concentration markedly with the hormonal status of the animal, means were sought to evaluate the contributions of various pathways to the utilization of 3-hydroxyanthranilic acid. C^{14}-labeled 3-hydroxyanthranilic acid was synthesized, carboxyl-labeled by the procedure of Ciereszko and Hankes, and ring-labeled (carbons 1 and 2) by a procedure involving the formation of phthalic anhydride from 2,3-labeled maleic anhydride, then conversion to phthalimide, anthranilic acid and 3-hydroxyanthranilic acid; the last step was hydroxylation with persulfate. The labeled substrates (about 1 mg/rat) were administered intraperitoneally. About 90% of the label from the carboxyl group appeared as CO_2 within the first several hours. The ring-labeled compound also gave rise to significant amounts of labeled CO_2; 20 to 40% was expired within 4 hr. Almost all of the remainder of the label was excreted in the urine within this time. Carboxyl-labeled nicotinic and picolinic acids gave rise to very little if any labeled CO when given in comparable doses. These results indicate that a large part of 3-hydroxyanthranilic acid metabolism cannot be acounted for by known reactions. It remains to be determined whether the degradation of this substrate includes the intermediary formation of the pyridine carboxylic acids. The identities of the labeled products found in the urine are being investigated.

950. ANTI-RENNIN AND ANTI-CHYMOTRYPSIN ACTIVITY OF SERUM. Jacklyn Melchior and Robert Sliwinski.* Depts. of Pharmacology and Biochemistry, Loyola Univ., Chicago, Ill.

The inhibition of the enzymic clotting of milk by small amounts of human serum has been demonstrated by West and Hilliard (Proc. Soc. Exper. Biol. & Med. 71:169, 1949), who also reported that sera obtained from cancer patients showed a decreased anti-rennin and increased anti-chymotrypsin activity when compared to normal samples. In the present study, the anti-rennin and anti-chymotrypsin activity of sera drawn from male albino rats has been studied, using the inhibition of hemoglobin proteolysis at pH 4.0 and of casein proteolysis at pH 7.6, respectively. A 1 hr pre-incubation of serum with rennin was required to demonstrate full inhibitor activity. Hypophysectomy caused an increase of 84% in anti-rennin activity and a decrease of 35% in the anti-chymotrypsin activity of the sera. Thus, removal of the pituitary prduces an effect which is opposite to that described as characteristic of malignant disease (loc cit). Replacement therapy showed ACTH, growth hormone and whole pituitary to be without effect on the anti-rennin activity of sera of hypophysectomized rats, while addition of 0.25% thyroxin to the diet caused a decrease to levels approaching those found in the intact animals. Growth hormone was most effective in increasing the anti-chymotrypsin level of the hypophysectomized animals; feeding thyroxin caused a slight decrease, whole pituitary a slight increase, while ACTH was without effect. These anti-proteolytic activities of serum thus appear to be closely related to the endocrine bablance of the host, with thyroxin and growth hormone acting as specific controlling factors.

951. ORGANIC ACID EXCRETION IN EXPERIMENTAL ALKALOSIS. Paul Melius* and Morris A. Lipton. VA Research Hosp. and Dept. of Medicine, Northwestern Univ. Med. School, Chicago, Ill.

It has been demonstrated by several workers and confirmed in this laboratory that animals in which alkalosis has been experimentally produced excrete large quantities of citric acid in their urine. It has also been reported (Cooke et al. Am. J. Med. 17:180) that citric acid represents only a fraction of the total organic acids excreted in this condition. However, no attempt was made by previous workers to characterize the other organic acids. In addition, the Van Slyke and Palmer method (J. Biol. Chem. 68:245, 1926) used by these workers for the determination of total organic acids does not give quantitative results. We have developed a method to quantitatively determine the total organic acids of urine and also to determine the individual acids separately. This was accomplished using anion exchange chromatography on Dowex 1 resin to separate organic acids from inorganic acids. Rats were made alkalotic by feeding a basal diet low in organic acids to which was added 10% sodium bicarbonate. Urines were collected quantitatively every 24 hr and were analyzed for total nonvolatile organic acids by elution from the Dowex 1 column with 4N formic acid, and for the individual acids by extended gradient chromatography. The results show an increase of 40-fold in the quantity of citric acid excreted during alkalosis (confirmed by direct chemical analysis) and an increase of about 15-fold in the total organic acid excreted. Citric acid represents about 50% of the total organic acid excreted. Data on the quantities of other organic acids excreted in alkalosis will be presented.

952. MECHANISM OF CARBAMYL PHOSPHATE SYNTHESIS IN LIVER PREPARATIONS. R. L. Metzenberg,* L. M. Hall* and P. P. Cohen. Univ. of Wisconsin, Madison.

The requirement for catalytic amounts of glutamate derivatives in the production of carbamyl phosphate by preparations from mammalian liver suggests that the mechanism is different from that operating in bacteria. In order to gain information on this point, dog liver has been fractionated to yield stable, active preparations which are virtually free of adenosine triphosphatase, myokinase and pyrophosphatase. This has made it feasible to conduct a stoichiometric study of carbamyl phosphate synthesis as a function of phosphate release. Carbamyl phosphate was measured by simultaneous conversion to citrulline in the presence of ornithine and a large excess of added ornithine-carbamyl phosphate transcarbamylase. It has been found that at rate-limiting concentrations of

acetylglutamate or carbamylglutamate 2 moles of inorganic phosphate are liberated per mole of citrulline formed. The nucleotide produced has been identified as adenosine diphosphate and is formed in amounts equivalent to the amount of inorganic phosphate liberated. In the presence of excess quantities of acetylglutamate, more than 2 moles of phosphate are liberated per mole of citrulline formed. These data, in addition to other evidence, are consistent with the existence of an intermediate prior to carbamyl phosphate in the biosynthesis of citrulline by liver.

953. C^{14} LYSINE INCORPORATION INTO BENCE JONES PROTEIN BY CULTURES OF MYELOMA BONE MARROW. Franz Meyer (intr. by Lloyd M. Kozloff). Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

Neoplastic plasma cells of the bone marrow have long been implicated as a site of synthesis of Bence-Jones protein (BJ) in persons afflicted with multiple myeloma, although recent attempts to demonstrate this experimentally have not been successful (Z. Naturforsch. 11b:588, 1956). In the present experiments, material aspirated from sternal marrow of a myeloma patient excreting large amounts of BJ was incubated for 72-168 hr in a modified Eagle's medium containing 50% human serum following the method of Steiner and Anker (Proc. Natl. Acad. Sci. U. S. 42:580, 1956). C^{14} lysine was used as tracer. The cell-free dialyzed material from the incubation mixture was fractionated by starch zone electrophoresis and essentially all the radioactivity was found in the 1 zone corresponding to gamma globulin (GG) and the BJ protein of this patient. The BJ protein could be distinguished from the GG by 3 independent fractionation procedures (using unlabeled BJ and human GG as carriers): 1) precipitation of a protein fraction that coagulated at 58° and redissolved at 100°; 2) specific antibody precipitation of human GG; and 3) differential centrifugation. In each case 91-95% of the activity resided in the portion exhibiting BJ protein characteristics.

954. REACTION OF IODINATED FIBRINOGEN WITH THROMBIN. Elemer Mihalyi. Natl. Heart Inst., Bethesda, Md.

Intermolecular hydrogen-bonding, involving tyrosine and histidine residues, has been proposed as the mechanism of polymerization of fibrinogen. Whereas the role of histidine appears well demonstrated, that of the tyrosine needs further support. Therefore, the effect of iodination of the tyrosine groups on the polymerization was investigated. Iodination at 0° and pH 8.6 in the presence of 20% urea involves tyrosine and histidine residues to about the same extent and slight oxidative side reaction is also apparent. The iodinated fibrinogen is able to polymerize, shown by an increase in viscosity and turbidity upon thrombin addition. The rate of these changes was determined as a function of pH and ionic strength. The pK-s of the iodinated residues are shifted toward more acidic values. A similar shift of the alkaline end of the pH vs. rate of polymerization curve was observed when more than half of the tyrosine residues were iodinated. This observation provides additional support for the participation of unionized phenolic OH groups in the bond formation. The iodinated fibrinogen samples are soluble in the absence of salt. This makes it possible to extend the investigation of the clotting reaction at low ionic strength. In the absence of salt, at pH 8, polymerization does not occur. Under these conditions, however, the primary action of thrombin is inhibited. The rate of polymerization at any given ionic strength is the same whether or not the iodinated fibrinogen has been preincubated with thrombin in the absence of salt. At this pH at least, the electrostatic repulsion between the fibrinogen and thrombin seems to be sufficient to prevent reaction. The rate is increased proportionately to the square of the ionic strength.

955. FAVORABLE EFFECT OF SODIUM IN AMINO ACID METABOLISM OF LACTIC ACID BACTERIA. John N. Mills, Wang Lim Chun, and Robert J. Sirny (intr. by V. G. Heller). Dept. of Agricultural Chemistry, Oklahoma Agricultural Exp. Station, Stillwater.

Inhibitory effects of Na^+ in media for lactic acid bacteria are well-known and have been attributed to an ion antagonism between Na^+ and K^+. However, certain beneficial effects of sodium have also been observed. In studies with Lactobacillus arabinosus, both favorable and unfavorable effects have been observed and some of these have been previously reported (Fed. Proc. 15:356, 1956). Further studies of the favorable effects of Na^+ in this organism have revealed that glycine utilization and synthesis are both improved in media containing relatively high Na^+ concentrations. Growth in the absence of serine is also improved, as is also the growth of Leuconostoc mesenteroides, under similar conditions. The interconversion of glycine and threonine, manifested by the growth of L. arabinosus in the absence of one or the other (but not both) of these amino acids, also proceeds more readily in Na^+-containing media. These beneficial effects of sodium, which are especially evident in the early growth phase of these bacteria, appear to be associated with specific conditions in which the concentrations of certain nutrients have been reduced to growth-limiting amounts or in which partial inhibition of either the utilization or synthesis of a nutrient is involved. The specific nutrient interrelationship cited, as well as other pertinent ones, will be discussed, and the possible implications of these favorable effects of Na^+ will be considered.

956. IN VITRO INCORPORATION OF PROLINE-C^{14} AS HYDROXYPROLINE-C^{14} IN COLLAGEN. Chozo Mitoma and Thomas E. Smith (intr. by Sidney Udenfriend). Natl. Heart Inst., Bethesda, Md.

Polyvinyl sponges (1.5 x 1.5 x 0.5 cm) were implanted subcutaneously in rats according to the procedure of Boucek and Noble, Arch. Path. 59:553, 1955. After 2 wk the fibroblast rich sponges containing about 10 mg of collagen were removed and minced with scissors. When the minced tissue from 1 sponge was incubated with 1 mg of L-proline-C^{14}, containing 1 x 10^6 cpm, at 37°, under oxygen and in the presence of a complete supplement of amino acids approximately 10,000-20,000 cpm were incorporated into the protein. Upon hydrolysis of the collagen, 5% of the incorporated radioactivity was found to be in the hydroxyproline. Some of the factors involved in this in vitro conversion of proline to hydroxyproline will be discussed.

957. METABOLISM OF GLUTAMINE IN HUMAN LIVER. Kivie Moldave* and Alton Meister. Dept. of Biochemistry, Tufts Univ. School of Medicine, Boston, Mass.

Human liver homogenates were incubated with randomly labeled C^{14}-L-glutamine in the presence and absence of phenylacetyl-CoA (prepared by reaction of CoA with phenylacetic anhydride). Chromatographic analysis revealed, in addition to C^{14}-glutamine, radioactive glutamate, α-ketoglutarate and pyrrolidonecarboxylate. In the presence of phenylacetyl-CoA, a radioactive product was formed, which

nowed with a synthetic sample of phenylacetylglutamine (PAG) in 5 paper chromatographic systems, and on column chromatography with Dowex-1-chloride. Isolation of this radioactive product followed by acid hydrolysis gave C^{14}-glutamic acid. In an attempt to elucidate the synthesis of PAG, human liver homogenates were incubated with L-phenylalanine, CoA, ATP, pyruvate, and C^{14}-glutamine. Chromatograms showed the presence of radioactivity in the PAG area; when C^{14}-glutamine and C^{14}-L-phenylalanine were employed, similar results were obtained. Omission of CoA or of ATP resulted in significantly lower conversion. Less conversion occurred when C^{14}-phenylacetate was substituted for C^{14}-phenylalanine. With rat liver preparations, no radioactivity was found in the PAG area. PAG, which probably represents an end-product of phenylalanine metabolism, is known to be present in human urine. It is also known that humans excrete administered phenylacetate in the urine as PAG, whereas phenylacetylglycine is characteristically excreted by other mammals. The metabolic pathways in man of phenylalanine and of glutamine therefore appear to be unique in this respect as compared with other mammals. The enzyme system that catalyzes the condensation of phenylacetyl-CoA and glutamine is present in the soluble fraction of human liver. The reaction has also been investigated by means of chemical and spectrophotometric procedures. Studies on the purification of the enzyme system have been carried out.

958. AMINO ACID UTILIZATION IN THE IRRADIATED RAT. Margaret G. Morehouse and Ronald L. Searcy.* Univ. of Southern California, Los Angeles.

A marked stimulation in liver glycogen formation has previously been demonstrated in X-rayed animals despite fasting (Science 112:158, 1955). The possibility that protein breakdown induced by irradiation may provide precursors for liver glycogen has been investigated. Glycine-1-C^{14}, alanine-1-C^{14} or leucine-1-C^{14} in microcurie amounts were injected intraperitoneally into fed and fasted normal rats and into those similarly treated but subjected to a whole-body irradiation dose of 1500 r. All animals were killed 2 hr after the administration of the amino acid. The incorporation of the isotopic glycine into hepatic glycogen was greater in the normal nonfasted rats than that observed with alanine or leucine. With the latter 2 amino acids fasting did not alter the itotopic levels in liver glycogen. However, in the case of glycine, fasting increased the C^{14} incorporation into glycogen some 3-4 times over that of fed aninals. Immediately following exposure an irradiation induced increase in total counts noted in the glycogen was evident with all the amino acids. The effect was most pronounced with the glycogenic amino acid, alanine and least observable with leucine. A similar relationship obtained in the irradiated rats after periods of fasting but here the levels of incorporation were much higher. It would thus appear that amino acids may serve as a carbon source for glycogen formation after irradiation regardless of their usual classification as glycogenic or ketogenic. The possible role of these amino acids in tissue lipogenesis in normal and irradiated rats will be discussed. (Supported by Atomic Energy Comm. contract AT (11-1)-113, Project 6.)

959. CHROMATOGRAPHY OF MOUSE PANCREAS RIBONUCLEASES. Gene A. Morrill* and Sherman R. Dickman. Dept. of Biological Chemistry, Univ. of Utah College of Medicine, Salt Lake City.

CBA mice were fasted 6-12 hr, then killed by severing the spinal cord. The pancreas was removed, incubated 2 hr at 38° in Krebs' III buffer with and without the addition of pancreozymin. The pancreas was extracted according to Hirs, Moore and Stein (J. Biol. Chem. 200:493, 1953), the extracts chromatographed over XE-64 resin and the eluates assayed for ribonuclease activity and amino nitrogen by a ninhydrin procedure. The media from the pancreozymin-treated samples was also chromatographed. The chromatographic elution pattern of the nonstimulated, incubated or nonincubated controls was quite different from that reported with beef pancreas extracts in that in addition to peaks corresponding to ribonucleases A and B the largest activity peak was much more slowly eluted. This peak had the highest specific activity of any of the peaks obtained from mouse pancreas. The pattern of the pancreozymin-stimulated tissue extracts lacked this slowly eluted peak and the greatest activity was found in the same elution range as peak B of beef pancreas. This peak was followed by a broad, irregular plateau of ribonuclease activity which demonstrated no major peaks. The incubation media contained 2 major peaks, corresponding in elution volume to beef pancreas peaks A and B, peak B being considerably larger than peak A. In contrast to the specific activities of these 2 peaks in the stimulated tissue, peak B had a much lower specific activity than peak A. In this respect the media containing the secreted ribonuclease resembles beef pancreas extracts. The activity of these various peak fractions from mouse pancreas versus ribonucleic acid "core" will be reported.

960. METABOLISM OF C^{14} MYO-INOSITOL IN THE RAT. E. A. Moscatelli* and J. Larner. Div. of Biochemistry, Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.

C^{14}-labeled myo-inositol has been prepared photosynthetically utilizing either Hevea or corn plants (leaves and stems of the former kindly supplied by J. Skok of Argonne National Laboratories) and isolated according to the method of Halliday and Anderson, J. Biol. Chem. 217:797, 1955. Fasted rats were injected intraperitoneally with C^{14} myo-inositol after preceding nonlabeled glucose injections. Animals were placed in closed circuit metabolism cages, CO_2 collected during a 12-1/2 hr time period and analyzed for radioactivity. At the end of the experiment animals were killed, liver glycogen isolated and crude phospholipds extracted from liver, brain, heart and kidney. Analysis revealed that respiratory CO_2 was radioactive to the extent of approximately 25% of the injected dose (uncorrected for urinary inositol excretion) indicating a vigorous overall oxidative dissimilation. Liver glycogen and tissue phospholipids were labeled to a much smaller extent. Glycogen labeling confirms previous demonstrations of conversion of myo-inositol to glucose. (Posternak et al. Helv. Chim. Acta 38:1283, 1955; Stetten and Stetten. J. Biol. Chem. 164:85, 1946.) (Aided in part by a grant from the Lipotropic Fndn.)

961. TITRATABLE SULFHYDRYL GROUPS OF FETAL AND HOMOZYGOUS C HEMOGLOBINS. Makio Murayama (intr. by Henry A. Sloviter). Dept. of Biochemistry, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

The hemoglobins were crystallized by the method of Drabkin (Arch. Biochem. 21:224, 1949), in 2.8 M phosphate buffer, pH 6.8. The crystals were dissolved in water and dialyzed against a large volume of deionized water. The amperometric-argentometric

method of Benesch, Lardy and Benesch (J. Biol. Chem. 216:663, 1955) and the amperometric-mercurimetric method of Kolthoff, Stricks and Moren (Anal. Chem. 26:335, 1954) were used. The experimental findings suggest that in fetal hemoglobin (Hb-F) there are about 6 titratable sulfhydryl groups (6 silver ions) per molecule at 0° and 38°C, whereas only 3 mercuric ions per molecule are bound by this hemoglobin at these temperatures. In homozygous C hemoglobin (Hb C/C) there are about 4 titratable (accessible) sulfhydryl groups at 0°, 4 silver and 4 mercuric ions are bound per molecule. At 38°C, on the other hand, Hb C/C binds about 8 silver and 8 mercuric ions per molecule. Conclusions drawn are that in Hb-F there exist 3 pairs of sulfhydryl groups, whereas in Hb C/C the sulfhydryl groups are so arranged that pairing does not occur.

962. ACYLATION AND DEACYLATION OF CARCINOGENIC FLUORENE DERIVATIVES BY RAT TISSUE. Herbert T. Nagasawa* and Helmut R. Gutmann. Radioisotope Service, VA Hosp. and Dept. of Physiological Chemistry, Univ. of Minnesota, Minneapolis.

Rat liver slices were incapable of utilizing exogenous butyrate or succinate for direct acylation of the carcinogen, 2-aminofluorene (AF), in a manner analogous to exogenous acetate utilization (J. Biol. Chem. 211:63, 1954). The masking effect of a rapid hydrolysis was ruled out since N-(2-fluoren-9-C^{14}-yl)-succinamate (SAF-9-C^{14}) was not hydrolyzed. The corresponding butyryl derivative, N-(2-fluoren-9-C^{14}-yl)-butyramide (BuAF-9-C^{14}), was, however, cleaved rapidly under these conditions. Nevertheless, the label on the isolated butyryl derivative after incubation of AF-9-C^{14} and butyrate with liver slices was so low as to exclude even a 1% net synthesis of BuAF-9-C^{14}. Further study of rat liver deacylase, as found in slices, disclosed a high degree of selectivity toward 2-acylaminofluorenes; thus, only the amides of AF derived from alkanoic acids were readily hydrolyzed. In contrast, intestinal deacylase exhibited less specificity and greater potency. The extent of deacylation of several 9-labeled 2-acylaminofluorenes by rat intestine in vitro was as follows: BuAF-9-C^{14}, 72.3%; N-(2-fluoren-9-C^{14}-yl)-acetamide, 38.1%; SAF-9-C^{14}, 30.9%; N-(2-fluoren-9-C^{14}-yl)-benzamide, 14.0%; N-(2-fluoren-9-C^{14}-yl)-p-toluenesulfonamide, 0.67%. These relative rates parallel roughly the carcinogenicity of these compounds. The data therefore implicate the intestine as the principal site for liberation of AF after ingestion of these derivatives. Evidence is presented that the hydrolytic reactions observed with intestine were due to an intestinal deacylase and not to bacterial action. (Supported by grant C-2571, Public Health Service.)

963. NITROGEN FIXATION BY SONICATES OF AZOTOBACTER. Alvin Nason, Hajime Takahashi,* George Hoch* and R. H. Burris. McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md., and Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Preparations obtained by sonic oscillation of Azotobacter vinelandii in the presence of sucrose and potassium phosphate catalyze the fixation of N_2 into a trichloroacetic acid-soluble fraction at 25 - 100% the rate of whole cells. The intact cells remaining in the sonicates ranged from 15 to 40% (maximal concentration as estimated turbidimetrically). Sucrose, which is necessary for N_2 fixation by sonicates and whole cells, enhances the rate 2- to 3-fold upon an increase in concentration from 2 to 10%. Added potassium phosphate (0.05 M) stimulated N_2 fixation 3- to 4-fold in sonicates and whole cells. Pyrophosphate results in a complete loss of activity by the sonicates with little effect on whole cells. The precipitate collected by centrifugation of the sonicate is responsible for the N_2 fixation while the supernatant solution shows no activity. The latter, however, stimulates activity of unwashed precipitates and inhibits that of whole cells. Succinate, malate and citrate (0.2 M to 0.3 M) were completely inhibitory to the precipitates, sonicates and whole cells. Pyruvate, also a strong inhibitor of precipitates and sonicates, had only a small effect on whole cells. The calculated relative N_2 fixation per remaining intact cell of the sonicate averages twice that of the control whole cells. It is not unlikely that an osmotically sensitive, large particle is responsible for N_2 fixation in the sonicate. This is supported by observations that relatively high concentrations of sucrose are necessary to obtain active sonicates, and that oscillation periods greater than 10 min yield inactive preparations.

964. SIMPLE PROCEDURE FOR DETERMINING THE EQUATIONS OF ABSORPTION SPECTRA AND CONCENTRATION VS. ABSORBANCE CURVES. Samuel Natelson and Stephen Ellis Natelson.* Rockford Mem. Hosp., Rockford, Ill.

It is customary to report absorption spectra by plotting absorptivity against the wave length. It would be advantageous if authors were to write the equation for the graph so that the value, at any particular wave length, could be determined accurately. This equation, in factorial form, can be readily obtained by a series of subtractions, using Newton's theorem in the calculus of finite differences. Equal intervals of wave length must be chosen assigning the numbers 0, 1, 2, 3, etc. to successive values. Let us take the absorption spectrum of urea as the diacetyl complex as an example. We now set up a "difference table."

Wave Length (mμ)	x	U_x	ΔU_x	$\Delta^2 U_x$	$\Delta^3 U_x$	$\Delta^4 U_x$	$\Delta^5 U_x$	$\Delta^6 U_x$	$\Delta^7 U_x$
400	0	22	43	84	41	-156	-499	2987	-8494
420	1	65	127	125	-115	-655	2488	-5507	
440	2	192	252	10	-770	1833	-3019		
460	3	444	262	-760	1063	-1186			
480	4	706	-498	303	-123				
500	5	208	-195	180					
520	6	13	-15						
540	7	-2							

(U_x = absorbance x 1000)

U_x are the values actually obtained (absorbance). ΔU_x is the 1st difference, obtained by subtracting the succeeding value from the first, (example, 65 - 22 = 43; 192 - 65 = 127). $\Delta^2 U_x$ is the 2nd difference obtained by subtracting successive values in the ΔU_x column as before. (Example: 127 - 43 = 84; 252 - 127 = 125.) This is continued until $\Delta^n U_x$ is obtained. In this case n = 7. The 1st value in each column is the coefficient of the factorial equation as follows. $U_x = 22 + 43x^{(1)} + \frac{84x^{(2)}}{2!} + \frac{41x^{(3)}}{3!} - \frac{156x^{(4)}}{4!} - \frac{499x^{(5)}}{5!} + \frac{2987x^{(6)}}{6!} - \frac{8494x^{(7)}}{7!}$. The numbers such as 3! (3 factorial) = 3.2.1. The powers are also factorial, thus $x^{(4)} = x(x-1)(x-2)(x-3)$. In applying this equation one must substitute simple whole or fractional numbers for x to obtain the unknown absorbance. For example, 400 mμ = 0, and 420 mμ = 1. Therefore 410 mμ = 0.5 and 405 mμ = 0.25.

965. METABOLISM OF TRANSPLANTED KIDNEY. P. Nathan,* and Benjamin F. Miller. May Inst. for Med. Research of Cincinnati Jewish Hosp. and Depts. of Physiology and Medicine, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

A technique has been devised for the transplantation of dog kidney from 1 animal to another without interrupting the blood supply to the organ. This has been accomplished by supplying the kidney with an accessory circulation from the donor animal while connecting the renal vessels to the host's vascular system. The usual method for transplantation subjects the kidney to a period of ischemia lasting 1/2 to 1 1/2 hr. The animals were killed at intervals up to 6 days following transplantation and the oxygen consumption, sodium and potassium content and the percentage water of kidney cortex were measured. In all instances there was a decrease in the oxygen consumption of the transplanted kidney when compared to its control. The transplanted kidney may double in size and weight within 2 days following the operation. The percentage water of the renal tissue does not change; the potassium to sodium ratio remains constant or rises. This suggests proportional increases in dry weight, intracellular and extracellular compartments. The metabolism of kidneys transplanted without interrupting their blood supply is maintained at a level closer to normal than those subjected to a period of ischemia. (Supported in part by the Josiah Macy, Jr. Fndn.)

966. VITAMIN B_{12} AND NUCLEIC ACID METABOLISM. Francis A. Nayder* and B. S. Schweigert. Biochemistry Dept. and American Meat Inst. Fndn., Univ. of Chicago, Chicago, Ill.

The relationship of B_{12} to nucleic acid metabolism has been investigated in Lactobacillus leichmannii 313 (ATCC 7830). In this organism the requirement for B_{12} can be replaced either by 1) the combined presence of an active purine and a deoxyriboside or 2) deoxyguanosine or deoxyinosine alone. The 2 essential requirements for B_{12} replacement are, therefore, a source of purine and a source of chemically combined deoxyribose. As a source of purine, guanine, xanthine and hypoxanthine are active, while adenine and the free base and riboside of 4-amino-5-imidazolecarboxamide are either inactive or partially active. The deoxyribosides of cytosine, thymine, adenine, and guanine appear to be equally active on a molar basis as a source of combined deoxyribose when they are tested in the presence of a mixture of adenine, guanine, xanthine and uracil. In addition, since the purine deoxyribosides of either hypoxanthine or guanine can replace B_{12}, pyrimidines appear to be nonessential supplements. The requirement for B_{12} has been characterized on the basis of the quantity required for half-maximum turbidity at 660 mu. In an experiment where the B_{12} requirement was 1350 UG/10 ml, a mixture of purine ribosides reduced this requirement to 80, uracil reduced it to 790, while a mixture of deoxycytidine and thymidine increased the B_{12} requirement somewhat. At present experiments are underway to determine the effects of graded levels of B_{12} on glycine incorporation into purines.

967. INFLUENCE OF PANTOYLTAURINE AND TAURINE ON THE HATCHING OF GOLDEN NEMATODE LARVAE. A. L. Neal (intr. by Louise J. Daniel). Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.

An unknown organic compound excreted by the roots of potato, tomato and other members of the Solonaceae family is known to stimulate hatching of Heterodera rostochiensis Wollenweber larvae. Calam, Todd and Waring (Biochem. J. 45:520, 1949) reported anhydrotetronic acid as an artificial stimulant with maximum activity at pH about 2.5. This compound was much less active than the natural substance and was relatively inactive at neutral pH. Pantoyltaurine at levels up to 50 - 100 μg/ml of solution was found to stimulate the emergence of golden nematode larvae. This stimulation was more pronounced in the presence of the natural stimulant found in leachings from tomato plants. Taurine was found to be very effective in stimulating hatchings whereas pantoyl lactone was ineffective. The stimulatory effect of pantoyltaurine is apparently due to the taurine portion of the molecule. At levels of 1 mμg and 10 mμg of taurine/ml of solution the average number of larvae per cyst hatched (50 - 100 cysts) increased from 38 to 68 and 76, respectively, after 10 days incubation at 20°C. From the data obtained it appears that taurine can be considered as a natural occurring hatching stimulant for the golden nematode.

968. HISTIDINE SYNTHESIS IN E. COLI. Amos Neidle* and Heinrich Waelsch. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.

Recently we have shown that the amide nitrogen of glutamine can act as a precursor of N-1 of the imidazole ring of histidine (J. Am. Chem. Soc. 78:1767, 1956). We now wish to report other precursors of imidazole ring nitrogen, with special reference to the origin of N-3. E. Coli was grown on minimal medium containing substrate in the presence of excess ammonia and the isolated histidine degraded as previously described. Aspartic and glutamic acid nitrogen was readily incorporated, the label appearing predominantly in N-3. When guanine 2-C^{14} labeled with N^{15} in positions 1, 3 and the amino group was used as substrate, 23% of histidine N-3 originated from N^{15} labeled positions of guanine. The atom ratio of carbon to nitrogen transferred was close to unity. With guanine labeled with N^{15} exclusively in the amino group (prepared from 2-S-methylhypoxanthine), no N^{15} was incorporated. These results indicate that N-1 and C-2 of guanine are utilized as a unit for positions N-3 and C-2 of histidine. This is consistent with the fact that aspartic acid nitrogen labels both N-1 of guanine and N-3 of histidine and with the finding of Mitoma and Snell that in L. casei C-2 of guanine contributes to C-2 of histidine. The experiments also show that the amide group of glutamine contributes to N-1 of histidine without prior incorporation into N-3 of a purine. The possibility that xanthine or a derivative, such as ureidoimidazole carboxylic acid, is involved in the group transfer is being investigated.

969. STRUCTURAL CHEMISTRY OF FERRICHROME. J. B. Neilands. Dept. of Biochemistry, Univ. of California, Berkeley.

The molecular weight of ferrichrome, the crystalline iron chelate compound from Ustilago sphaerogena (J. Am. Chem. Soc. 74:4846, 1952) has been established by direct physical measurement to be 725 ± 25. The iron atom is in the ferric state and is bound to the organic part of the molecule by weakly covalent or ionic forces, some of which are enolic in character. Ferrichrome contains several residues of glycine and 1 of L(+) ornithine per mole. There is evidence that at least 1 residue of glycine is bound, via an amino group, to the iron atom. Partial acid hydrolysates of ferrichrome contain glycine, glycyglycine and various other unidentified ninhydrin-positive materials. Total hydrolysis yields glycine and ornithine as well as several residues of acetic acid and ammonia.

Ferrichrome is thus a ferric chelate derivative of a heteromeric peptide.

970. FORMATION AND INTERCONVERSION OF SUGAR NUCLEOTIDES BY PLANT EXTRACTS. E. F. Neufeld,* V. Ginsburg,* E. W. Putman,* D. Fanshier* and W. Z. Hassid. Dept. of Agricultural Biochemistry, Univ. of California, Berkeley.

Extracts from mung been seedlings and from numerous other plants contain pyrophosphorylases which, in the presence of a divalent cation (Mg^{++}, Mn^{++}, or Co^{++}), catalyse the reaction:

UTP + sugar-1-phosphate $\rightleftharpoons$ UDPsugar + PP.

The sugar phosphates which have been found to participate in the reaction include the 1-phosphoric acid esters of the following pyranoses: α-D-glucose, α-D-galactose, α-D-xylose, and both α- and β-L-arabinose. These give rise to UDPglucose, UDPgalactose, UDPxylose, and UDParabinose, respectively. The 1-phosphoric acid esters of β-D-glucopyranose, β-D-galactopyranose, β-D-xylopyranose, α-D-arabinopyranose, α-D-arabinofuranose, α-L-arabinofuranose, β-L-arabinofuranose and D-fructose, were not incorporated into sugar nucleotides. Extracts from mung bean seedlings are also able to catalyse the interconversion of UDPglucose and UDPgalactose, and of UDPxylose and UDParabinose. (Supported in part by a research grant from the Corn Industries Research Fndn. and a contract with the Atomic Energy Comm.)

971. OXIDATION OF CERTAIN THIO COMPOUNDS BY PREPARATIONS FROM PIRICULARIA ORYZAE. Harold A. Neufeld, Lonzo F. Green, Frances N. Latterell and Robert L. Weintraub (intr. by Milton W. Slein). Fort Detrick, Frederick, Md.

In the course of further investigation of the polyphenolases of the rice blast fungus, Piricularia oryzae, (Fed. Proc. 15:320, 1956) several classical inhibitors of copper-containing enzymes were employed. Low concentrations of cyanide and azide were highly effective, whereas high concentrations of sodium diethyldithiocarbamate (DEDTC) not only failed to inhibit but, in some cases, stimulated oxygen consumption. It has been found that preparations from P. oryzae contain an enzyme capable of oxidizing DEDTC to tetraethylthiuram disulfide. Sodium dimethyldithiocarbamate, several xanthates and thioacetic acid also were oxidized by this enzyme whereas cysteine, glutathione, thiourea, thioglycolic acid, thioglycerol and mercaptosuccinic acid were not. The oxidation of DEDTC was inhibited by cyanide and azide. Hydrogen peroxide does not appear to be produced in the reaction. Various methods for measuring the activity of this enzyme have been devised.

972. DEGRADATION OF TOBACCO MOSAIC VIRUS BY ALKANOLAMINES. Philip Newmark and Richard W. Myers (intr. by R. C. Mills). Dept. of Biochemistry, Univ. of Kansas, Lawrence.

Amino alcohols having the general formula R'R''CNH CH OH are singularly effective in degrading tobacco mosaic virus (TMV) into its protein and nucleic acid moieties. Ionic strength, pH and temperature of the buffer, protein concentration and age of the solution, all affect the degree of aggregation of the protein degradation product. In these respects the virus protein behaves like the nucleic acid-free abnormal protein which also appears in TMV-infected tobacco plants (Abstr. 125th Am. Chem. Soc. Meetings, Mar. 1954, p. 9C). With alkanolamine buffers, kinetic studies on TMV degradation can be carried out under conditions where no reaggregation of the degraded protein occurs. Dialysis of a 1% TMV solution against 0.02 M ethanolamine at pH 10.5, for 24 hr, at 0°-5°, yields a single, symmetrical, low molecular weight peak in the ultracentrifuge. Only traces of high molecular weight residues of virus size can be detected. Although the protein subunit obtained by this procedure is smaller than Schramm's 100,000 m.w., 4 S "A-protein" (Z. Naturforsch. 10b:481, 493, 1955), it still recombines with TMV nucleic acid to give infective virus-like particles. The relationship of this protein to the physical and chemical subunits of the virus protein will be discussed.

973. SERIAL CHANGES IN TISSUE CO_2 DURING ACUTE RESPIRATORY ACIDOSIS. George Nichols, Jr. (intr. by Eric G. Ball). Depts. of Medicine and Biological Chemistry, Harvard Med. School, Boston, Mass.

The distribution of CO_2 in the blood, muscle, brain and bone of rats was studied following exposure to 24% CO_2 in air. Groups of rats were killed after 1/2, 1, 3, 5, 7, 15, 24 and 48 hr of exposure. Measurements of total tissue, H_2O, fat, CO_2, Cl^- and blood pH were made. Blood pH fell in the first 1/2 hr from 7.47 to 7.05. pCO_2 in the blood rose within 1/2 hr to 160 mm Hg and remained there. Total CO_2 in blood, muscle and brain rose more slowly, reaching a plateau at 7 hr. In contrast, total bone CO_2 declined from 84.9 to 75.7 mM/100 gm of dry solids. After the 15th hr plasma pH rose slowly due to increased serum bicarbonate, reaching 7.35 at 48 hr. Muscle CO_2 did not change while brain CO_2 rose slightly. Bone CO rose gradually to 81.6 at 48 hr, approaching the control value. Calculations of intracellular bicarbonate and pH revealed that muscle fiber and brain cell HCO_3^- rise asymptotically reaching a plateau at 7 hr. This slow rise in HCO_3^- compared to the rapid rise in pCO_3 resulted in a fall of intracellular pH from 7.20 to 6.55 in the first 1/2 hr followed by a gradual increase in 7 hr to 6.90 (muscle) and 7.00 (brain). Between the 15th and 48th hr cell pH increased slightly but less than plasma. The existence of CO_2 in bone as $CO_3^=$ rather than HCO_3^- explains the paradoxical decrease in bone CO_2. (Aided by Grant A-854, Public Health Service.)

974. CYTOCHROME c PHOTO-OXIDASE OF SPINACH. R. H. Nieman* and Birgit Vennesland. Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

Digitonin extracts of washed spinach chloroplasts, prepared in 0.35 M NaCl, contain an enzyme complex which catalyzes the oxidation of reduced cytochrome c on illumination. This photo-oxidase from spinach resembles the bacterial cytochrome c photo-oxidase studied by Vernon and Kamen. The spinach photo-oxidase is heat-labile and dependent on oxygen. It is inactive toward reduced dichlorophenol-indophenol and can be distinguished from a dark cytochrome c oxidase, which is also present, by the use either of cyanide or of high concentrations of inorganic salts. These reagents inhibit the dark oxidase. The inorganic salts also have a marked accelerating effect on the photo-oxidase. The digitonin extracts have been fractionated into 2 components, neither of which shows appreciable cytochrome c photo-oxidase activity when tested separately. Both of these components are heat-labile. Under appropriate conditions, the rate of the reconstituted cytochrome c photo-oxidase reaction is proportional to the product of the concentrations of the 2 components, each of which may therefore be used to assay the other. One component can be precipitated from the digitonin extract by addition of ethanol and resolubilized in 1%

digitonin. This component contains all of the chlorophyll present in the digitonin extract. The other component is water soluble and considerable purification of this 2nd component has been achieved by a variety of procedures commonly used for the fractionation of proteins. The 2nd component is stable to prolonged dialysis and contains no chlorophyll, but the possibility that there is another pigmented prosthetic group has not been excluded.

975. HEXOSE UPTAKE IN ASCITES TUMOR CELLS. Marshall W. Nirenberg and James F. Hogg (intr. by Joseph P. Chandler). Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.

In Ehrlich ascites tumor cells no glycogen is found nor can glycogen synthesis be demonstrated at high levels of glucose. Previous work (Nirenber and Hogg. J. Am. Chem. Soc. 78:6210, 1956) has indicated that a step prior to hexokinase action exists in these tumor cells. New experiments with Gardner lymphosarcoma cells have yielded like results. Manometric measurements of anaerobic glycolysis demonstrated that both glucose and fructose were utilized by the tumor cells whereas galactose was not. Galactose markedly inhibited fructolysis in intact cells, but had no effect upon glucolysis. In tumor homogenates anaerobic glycolysis proceeded at approximately the same rate as in intact cells. In sharp contrast to the results obtained with intact cells, however, galactose did not inhibit fructolysis. Experiments with 2-deoxy-D-glucose have shown that, although this compound is an effective inhibitor of glucose uptake, the phosphorylated derivative which forms is an equally effective inhibitor of phosphohexoisomerase. The hexokinase activity of clarified tumor homogenates was assayed by spectrophotometric determination of TPN reduction in the presence of glucose-6-phosphate dehydrogenase and phosphohexoisomerase. Details of this method will be presented. Under these conditions galactose was found to have no inhibitory effect whatsoever upon tumor hexokinase. Galactose and fructose must therefore compete for a site prior to the hexokinase step in both Gardner lymphosarcoma and Ehrlich carcinoma ascites tumor cells. Experiments with isolated cell membranes from the Ehrlich tumor are now in progress. (Supported in part by American Cancer Society Institutional Grant 22C.)

976. INVESTIGATION OF ANTIBODY-HAPTEN INTERACTION BY COMPETITIVE BINDING. Alfred Nisonoff* and David Pressman. Dept. of Biochemistry Research, Roswell Park Mem. Inst., Buffalo, N. Y.

The methods ordinarily used for investigating antibody-hapten equilibria are: a) quantitative estimation of the ability of small molecules to inhibit the precipitin reaction; b) direct binding measurements (equilibrium dialysis). Each method has important limitations. The latter is generally not practical for haptens with K values below $\sim 5 \times 10^3$. The former technique provides relative combining constants over a wide range of K values, but is not reliable for estimating other thermodynamic parameters because of uncertainty as to the composition of the soluble complexes formed in the presence of both antigen and hapten. This paper describes results obtained by a method of competitive binding, which permits the investigation, in the absence of antigen, of haptens having low K values. The ability of such haptens to displace a strongly bound hapten from the antibody is measured by equilibrium dialysis. Specifically purified antibody homologous to the p-azobenzoate haptenic group was studied. To check the method, the equilibrium constants for 2 strongly bound haptens, radioactive p-iodobenzoate and p-(p-hydroxyphenylazo)-benzoate, which is colored, were measured directly, and their relative constants were estimated by competitive binding. The results of the two methods agreed closely. The K values of several weakly bound haptens were also determined and found to be in good agreement with older data obtained by the method of hapten inhibition. An index of heterogeneity for the antibody and the number of combining sites per antibody molecule were also evaluated.

977. HORMONE-STIMULATED CONCENTRATION OF AN UNMETABOLIZABLE AMINO ACID. Matthew W. Noall* and Halvor N. Christensen. Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor, and Dept. of Obstetrics and Gynecology, Washington Univ., St. Louis, Mo.

The possibility is under consideration that certain hormones modify growth and catabolism by modifying the extent to which amino acids are concentrated by various tissues, thereby possibly facilitating synthetic or degradative reactions. Such changes in the distribution of a naturally-occurring amino acid are not easily distinguished from actions upon the synthetic or degradative reactions themselves. Accordingly carboxyl-labeled α-aminoisobutyric acid has been synthesized from acetone, NH_4Cl and $NaC^{14}N$. This amino acid is not degraded metabolically; after its injection into the rat no $C^{14}O_2$ expiration could be detected. Because it is inert and yet is concentrated by various tissues, it is slowly eliminated and its distribution may be studied several days after injection. Rats, 180 - 220 gm, were injected intraperitoneally with a tracer amount of the amino acid representing 5.3×10^5 cpm. Twenty-eight hours later 1 unit of growth hormone or (using adrenalectomized animals) 0.1 mg/kg of epinephrine were likewise injected. The animals were killed 2 hr later; the liver, kidney, muscle, heart, lung, duodenum, uterus + ovaries and brain were homogenized with dilute acetic acid and the extracts heated to deproteinize them. Counting was with a thin-windowed gas-flow counter. The extent to which the amino acid was concentrated was increased in all 8 tissues by epinephrine, for 7 out of the 8 by growth hormone, although at the present writing with only 4-7 animals per group some of the P values for individual tissues remain as high as 0.1.

978. BACTERIAL UTILIZATION OF PEPTIDES. William E. Nutter and Damon C. Shelton (intr. by R. F. Krause). Dept. of Agricultural Biochemistry, West Virginia Univ., Morgantown.

Certain peptides permitted a greater growth rate of Leuconostoc mesenteroides, than was afforded by comparable concentrations of their component amino acids. This phenomenon was most pronounced during the early exponential growth phase and at limiting levels of the peptide and amino acids. As the concentration of amino acids was increased, the growth rate approached that which was supported by the peptide and no difference was apparent when the level of amino acids had been increased 50-fold. The effect is on growth rate rather than total growth, since extended incubation reduced an initial, striking difference in growth. Cells were incubated with the most effective peptide, glycylglycine and subsequently ruptured and chromatographed. No glycylglycine could be detected in the cellular fluid, however, the peptide had been hydrolyzed and glycine was concentrated inside the cell. Other free amino acids found in the cellular fluid were alanine, serine, aspartic and glutamic acids. Apparently, glycylglycine was rapidly hydrolyzed prior to entry or at time of entry into the cell. Since no exocellular peptidases

could be detected in the supernatant after removal of the bacterial cells, it appeared that hydrolysis was occurring during entry into the cell. Several peptides both stimulatory and nonstimulatory were tested to determine their rate of hydrolysis by the bacterial cells. It was found that a direct relationship existed between the rate of peptide hydrolysis and the growth stimulatory activity. Thus, the amino acids of a stimulatory peptide appeared to be gaining entry into the cell by some mechanism involving hydrolysis. Subsequently, it was found that a stimulatory peptide was inhibited by another stimulatory peptide but not by a nonstimulatory one.

979. SYNTHESIS OF RNA BY POLYNUCLEOTIDE PHOSPHORYLASE. Severo Ochoa, Sanae Mii,* Morton C. Schneider,* R. M. S. Smellie,* Robert C. Warner and Priscilla J. Ortiz.* Dept. of Biochemistry, New York Univ. College of Medicine, New York City.

Ribopolynucleotides synthesized from nucleoside-5'-diphosphates by polynucleotide phosphorylase have the structural features, molecular weight, and other properties of RNA (Fed. Proc. 15:833, 1956). From equimolar amounts of the 4 naturally occurring nucleoside diphosphates purified polynucleotide phosphorylase of Azotobacter vinelandii catalyzes the synthesis of RNA of similar nucleotide composition and molecular weight as natural RNA isolated from Azotobacter. The conclusion that the biosynthetic compound is RNA is further supported by the finding that its ribonuclease-resistant residue stimulates formation of streptolysin S by Streptococcus haemolyticus as natural RNA does (F. Egami, personal communication) and by the fact that in samples of relatively undegraded natural RNA (Axotobacter, yeast) the same end-group structure is found as in the biosynthetic polynucleotides, namely phosphate monoesterified at carbon 5' of the nucleoside moiety. These results suggest that polynucleotide phosphorylase is concerned with the intracellular synthesis of RNA.

980. SPECIFIC P^{32} RADIOACTIVITY OF ACID-SOLUBLE COMPONENTS OF u.v. INDUCED AND T_2r INFECTED BATERIA. James F. O'Donnell,* R. P. Mackal* and E. A. Evans, Jr. Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

Comparison of the specific radioactivity of the DNA of purified lambda virus with that of acid soluble components of u.v. induced E. coli K12 indicates that the monophosphorylated nucleotides account for the specific radioactivity of the viral DNA. P^{32} was added to cells in fresh synthetic media, immediately after induction with ultraviolet light. With E. coli B, P^{32} was added at the same time as infection with T_2r. The induced cultures were aerated at 37°C for 45 min (about 1/2 the latent period) while the T_2r infected cultures were aerated for 15 min at 37°C. After centrifugation and washing with Ringer's solution, perchloric acid extracts were chromatogramed on Dowex-1. Gradient elution was carried out with formic acid-ammonium formate. In the u.v. induced system adenosine and cytidine monophosphate and other monophosphorylated components had as high or higher specific radioactivity than the isolated virus, while several of the more highly phosphorylated compounds did not. However, one as yet unidentified component, which was eluted later than the monophosphorylated compounds, did contain a high specific activity. In induced bacteria washed with Ringer's solution, a considerable amount of high labeled orthophosphate is present in the acid-soluble fraction and it appears that within the bacterial cell, orthophosphate is the primary source of virus nucleic phosphorus. Several non-ultraviolet light absorbing components also appear in the acid-soluble fraction. With respect to absorption on charcoal they behave as inorganic phosphate, but are eluted at different concentrations of formate-formic acid from Dowex-1. Most of them contain radioactivity comparable to that found in purified virus nucleic acid. In the case of E. coli B, normal and infected with T_2r, all the nucleotides contained some P^{32}. In the infected system the deoxynucleotides, which do not appear in the uninfected cells, also contain radioactivity.

981. MECHANISM OF ASCORBIC ACID-INDUCED OXIDATION OF EPINEPHRINE AND NOREPINEPHRINE. M. Jane Oesterling (intr. by Marion Fay). Woman's Med. College of Pennsylvania, Philadelphia.

Ascorbic acid (AH_2) at 10^{-3} M accelerates the rate of loss of the epinephrines from acetate buffered solutions of pH 4 or 5 (Fed. Proc. 15:322, 1956; Biochim. et biophys. acta, in press). Experiments herein reported indicate that the hydrogen peroxide formed during copper-catalyzed atmospheric oxidation of AH_2 is responsible in large part for this effect since substitution of 10^{-3} M H_2O_2 for AH_2 produced at pH 4 a comparable rate of loss. This effect was markedly accelerated by addition of 10^{-4} M $Cu(OAc)_2$ or CuCl. In absence of H_2O_2 addition of 10^{-4} M cuprous, cupric, or ferrous salts had very little effect at this pH. Extremely rapid destruction was produced at pH 4 by 10^{-4} M $FeSO_4$ plus 10^{-3} M H_2O_2 (Fenton reaction.) A slow Fenton type of reaction is believed to be responsible in large part for the adverse effect of AH_2 on the stability of the epinephrines in acid solution, the rate depending upon the trace amounts of copper (or iron) contaminating the solutions. The metal may be reduced to the lower valence state by AH_2, by endiol-containing decomposition products of dehydroascorbic acid (A), or by H_2O_2. A Fenton type of reaction in the presence of AH_2 produces the free radical intermediate between AH_2 and A; viz. AH. The latter is believed to be the actual oxidizing agent when excess AH_2 is present (Kauffmann. J. Am. Chem. Soc. 73:4311, 1951) and there is insufficient oxygen readily available to oxidize AH to A.

982. SHORT-CHAIN MONOETHENOID FATTY ACIDS AS POSSIBLE PRECURSORS IN BACTERIAL BIOSYNTHESIS OF CIS-VACCENIC ACID. William M. O'Leary* and Klaus Hofmann. Biochemistry Dept., Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

Investigations in this laboratory have demonstrated that cis-vaccenic, palmitic and lactobacillic acids are the major fatty acid components of Lactobacillus arabinosus, Lactobacillus casei and Lactobacillus delbrueckii. Minor proportions of palmitoleic, capric, lauric, myristic and stearic acids are also present. Evidently these lactobacilli have the ability to biosynthesize fatty acids since the media employed for their cultivation were essentially lipide-free. The presence of cis-vaccenic and lactobacillic acids as major fatty acid components of lactobacilli, and the observation that these acids can substitute for biotin in their nutrition seem to link biotin with the biosynthesis of cis-vaccenic acid. The mechanism of this biosynthesis is obscure. Since most experiments demonstrating a sparing action on biotin have employed long-chain monounsaturated fatty acids, it became of interest to test the ability of shorter unsaturated fatty acids to substitute for biotin. Palmitoleic, 7,8-

tetradecenoic, 5,6-dodecenoic, 3,4-decenoic, 3,4-octenoic, 3,4-hexenoic, crotonic, isocrotonic and vinylacetic acids were investigated and a number of these acids exhibited marked growth promoting activity for L. arabinosus, L. casei and L. delbrueckii. Certain saturated fatty acids inhibit the biotin-like activity of the short-chain unsaturated fatty acids. Thus the ability to stimulate bacterial growth in the presence of suboptimal quantities of biotin is not limited to longer chain fatty acids. The growth-promoting activity of some short-chain unsaturated acids suggests their possible role as intermediates in the biosynthesis of cis-vaccenic and lactobacillic acids in the organisms studied. Biosynthesis of cis-vaccenic acid through desaturation of long-chain saturated acids seems unlikely since these acids have no growth-promoting activity and since the bacteria studied appear to lack suitable dehydrogenase systems.

983. POLYNUCLEOTIDE PHOSPHORYLASES. Peter S. Olmsted (intr. by Richard H. McCoy). Biochemistry Dept., Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

Following the initial discovery of in vitro polynucleotide synthesis by Ochoa we have studied similar reactions using enzymes obtained from Micrococcus lysodeikticus. After cell growth and lysis, ethanol fractionation provided a stable and active, but insoluble preparation. Digestion with crude "trypsin," but not crystalline trypsin or chymotrypsin, solubilizes the nucleotide phosphorylase and completely destroys the contaminating myokinase. Further fractionation with ethanol and ammonium sulfate leads to approximately a 100-fold purification (the best preparation polymerizes about 20 μM ADP/mg protein/90 min). The various purified fractions are unstable, apparently because of oxidation of SH groups. Activity toward CDP and UDP is also present in the crude suspension, but is nearly or completely absent in purified preparations. This is evidenced by 1) absence of a nonmigrating spot at the origin in paper chromatograms; 2) absence of a soluble, ultraviolet absorbing precipitate after perchloric acid addition to the incubate; and 3) negligible formation of inorganic phosphate. Attempts are in progress to prepare UDP- and CDP-phosphorylases free of ADP-phosphorylase. Treatment of the enzyme with ribonuclease, followed by dialysis and Dowex-1-glycine adsorption, produces a preparation containing little if any nucleic acid (absorption ratio 280 mμ/260 mμ = 1.66) but retaining a large proportion of the original enzymatic activity. The addition of boiled crude enzyme (containing bacterial RNA) or yeast RNA did not increase the potency. Thus it is unlikely that a "primer" of preformed RNA is needed for activity.

984. PURIFICATION AND PROPERTIES OF YEAST ISOCITRIC LYASE. James Allen Olson (intr. by George K. Davis). Dept. of Biochemistry, J. Hillis Miller Health Ctr., Univ. of Florida, Gainesville.

The enzyme isocitric lyase, which cleaves d-isocitrate to glyoxylate and succinate, has been found in yeast and molds (Olson. Nature 174:695, 1954) and in bacteria (Smith and Gunsalus. J. Am. Chem. Soc. 78:5002, 1954; Sax. Biochem. J. 58:xx, 1954). An extremely sensitive assay system for the cleavage reaction has been developed which depends on the 250 mμ absorption of the formed glyoxylic acid semicarbazone. Neither semicarbazide nor any of the other reactants absorbs appreciably at this wavelength. Linear relationships were observed between either enzyme concentration or reaction time and 250 mμ absorption. By the use of this assay method, the enzyme has been purified 80-fold from bakers' yeast through a series of fractionation steps involving autolysis, heating, ammonium sulfate, protamine and ethanol. As in the case of the bacterial enzyme, cysteine (or glutathione) and magnesium are necessary cofactors. The Michaelis constants for the various reactants and other properties of the system have been determined for the purified enzyme. The mechanism of this reaction is being investigated.

985. BIOLOGICAL TURNOVER OF PYRUVATE-2-C^{14} IN DOGS AND MAN. Robert E. Olson, Anita Rau,* Susan Maury* and Beverly Stewart.* Dept. of Biochemistry and Nutrition, Grad. School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

Pyruvate-2-C^{14} (10 μc/mM) was administered intravenously to dogs and man as the sodium salt in doses of ca. 50μM/kg and the time course of specific radioactivities of blood pyruvate, lactate, glucose and carbon dioxide followed over a period of 6 hr. Pyruvate was isolated as the 2,4-dinitrophenylhydrazone, lactate as the dimedon derivative of acetaldehyde derived from carbons 2 + 3, glucose as the osazone and carbon dioxide as barium carbonate. The specific activity of blood pyruvate declined to negligible values in 5 min. and the biological half-life of blood pyruvate calculated from the linear semilogarithmic decay curve was 1.5± 0.2 min in both dogs and man. The degree and rate of dilution of the administered isotopic pyruvate suggested a rapid uptake of this substrate by cells and a distribution in total body water. The rate of turnover (ca. 3-5 mg/kg/min) of blood pyruvate is of the same order as that of blood glucose. Maximum specific radioactivity appeared in blood lactate 1 min after administration of the isotopic pyruvate and declined to negligible values over a period of 1 hr. The specific activity of respiratory carbon dioxide reached a maximum in 20-25 min and 45-55% of the total administered dose was excreted as $C^{14}O_2$ in 6 hr. Negligible activity was detected in the blood glucose. (Supported in part by the Public Health Service (A-620) and by the Dept. of Health, Commonwealth of Pennsylvania.)

986. TRANSFER OF ERGOTHIONEINE TO NEWBORN AND WEANLING RATS. Joseph A. Ontko* and Paul H. Phillips. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Ergothioneine in the animal body has a dietary origin. In this study the transfer of ergothioneine to the newborn and weanling rat was investigated. During this experiment 15 mature, female rats were fed a ration in which oats, corn and whole liver powder contributed a substantial level of ergothioneine. After 1 month on this diet, the female rats were bred. To prevent the young rats in the litters from consuming the maternal diet, the mother rats were fed at intervals in a separate cage during the last week of the lactation period. Ergothioneine in the maternal blood was determined at parturition and at weaning. Concurrently, blood samples from the newborn and weanling rats were analyzed for ergothioneine content. The maternal blood and the blood of the newborn contained 8.3 ± 0.9 mg% and 2.0 ± 0.2 mg% of ergothioneine. At weaning time the maternal blood and the weanling blood contained 4.5 ± 0.5 mg% and 7.4 ± 0.8 mg% of ergothioneine, respectively. The maternal blood ergothioneine thus decreased during lactation while the ergothioneine in the blood of the suckling rats rose. Ergothioneine therefore appears to cross placental membranes and is secreted in the milk of rats.

987. HYDROXYMETHYL TETRAHYDROFOLIC DEHYDROGENASE. M. J. Osborn and P. T. Talbert (intr. by B. Gabrio). Dept. of Biochemistry, Univ. of Washington, Seattle.

Extracts of beef or pigeon liver acetone powders contain the enzyme, hydroxymethyl tetrahydrofolic (hFH_4) dehydrogenase, which carries out the TPN-linked reaction: $hFH_4 + TPN^+ \rightleftarrows f^{10}FH_4 + TPNH + H^+$. The enzyme has been purified by precipitation at pH 5.4, by precipitation with neutral ammonium sulfate (30-45%); by precipitation with ethanol or acetone, and by adsorption and elution from calcium phosphate gel. The enzyme is not identical with the GSH-requiring, DPN-linked HCHO dehydrogenase described by Strittmatter and Ball. The substrate is hFH_4 (probably the h^{10} derivative), which can be obtained either by the interaction of HCHO and tetrahydrofolic acid (FH_4) or from L-serine and FH_4 in the presence of serine hydroxymethylase. The reaction product is N^{10}-formyl tetrahydrofolic acid ($f^{10}FH_4$), identified by absorption spectrum (λ_{max} at 258 and 300 mμ) and by conversion upon acidification to anhydroleucovorin ($f^{5,10}FH_4$). In Dowex-treated pigeon liver preparations there is a stoichiometric agreement between the amounts of $f^{10}FH_4$ and TPNH formed in the reaction. The equilibrium position of the reaction can be reversed by adding the components of the glucose-6-phosphate dehydrogenase system to trap TPN. Under these conditions $f^{10}FH_4$ is converted to hFH_4 (λ_{max} 295 mμ), and this product appears to be identical spectrally with that obtained from the serine hydroxymethylase reaction or from the interaction of HCHO and FH_4.

988. KINETICS OF CYTOCHROME b REDUCTION IN RAT HEART SARCOSOMES IN SHOCK. Lester Packer (intr. by Elizabeth R. B. Smith). Dept. of Biochemistry, Walter Reed Army Inst. of Research, Washington, D. C.

The efficiency of the phosphorylation processes in heart muscle of animals in shock is of interest because the failure of energy metabolism is one of the factors which has been implicated in irreversibility in shock. As an approach to this problem, shock was experimentally produced in male rats of the Sprague-Dawley strain by the Noble-Collip drum and by tourniquet techniques. The heart sarcosomes of shocked and normal animals were prepared by the method of Cleland and Slater (Biochem. J. 53:547, 1953), and the respiratory pigments in the intact sarcosomes were investigated with the aid of spectrophotometric methods. From oxidized-minus-reduced spectra the amount, and relative concentrations of cytochromes a, a_3, c, b, and flavoprotein in the two sarcosome preparations were calculated and found similar. When the cytochrome content of the two preparations was the same, the rate of succinate respiration in "shocked" sarcosomes was more rapid than in normal preparations, suggesting that phosphorylation processes were uncoupled from oxidation. A study of the effect of ADP upon the kinetics of cytochrome-b reduction by succinate in untreated and in azide-treated sarcosomes revealed that the degree of respiratory control in "shocked" sarcosomes was much poorer than in normal preparations. Experiments on the incorporation of P^{32}-labeled inorganic phosphate into ATP during succinate oxidation in normal and in "shocked" mitochondria confirmed these results. Special attention was directed to the effect of azide, ADP, 2,4-dinitrophenol, and antimycin-A on the steady-state of cytochrome-b reduction by succinate in normal and "shocked" sarcosomes.

989. CHROMATOGRAPHIC STUDIES ON THE TRANSFORMING DNA OF PNEUMOCOCCUS (PnDNA). Herbert B. Pahl,* Sam M. Beister and Aaron Bendich. Sloan-Kettering Inst. for Cancer Research, and Dept. of Microbiology, College of Physicians and Surgeons, Columbia Univ., New York.

Chromatographic fractionation procedure utilizing the anion-exchanger ECTEOLA (J. Am. Chem. Soc. 77:3671, 1955) has been applied to PnDNA to determine whether this chromatographic method alters the nucleic acid. A solution of PnDNA possessing streptomycin, penicillin and mannitol transforming activities (Proc. Natl. Acad. Sci. 40:55, 1954) was passed through a column of the exchanger with quantitative retention. The PnDNA was eluted with NaCl of increasing concentration followed by solutions of increasing pH. Several chromatographic fractions were thus obtained which exhibited considerable variation (from 0.1 to 5 times that of the original) in the specific streptomycin activity (transformants per μg DNA). There was essentially a quantitative recovery of the nucleic acid as well as of the transforming activities which indicated that no significant denaturation of the PnDNA had accompanied its chromatography on ECTEOLA. Moreover, the same number of transformants were obtained when an excess of the original PnDNA or of any column fraction was assayed, despite the 50-fold variation in specific activity. This also would tend to exclude partial degradation as an explanation for the low specific activity of certain fractions. The results of the fractionation of PnDNA support the concept that DNA is a mixture of different molecules.

990. THE PRESENCE OF AN ε-LYSINE ACYLASE IN ANIMAL TISSUES. W. K. Paik, Leah Bloch-Frankenthal, Sanford M. Birnbaum, Milton Winitz and Jesse P. Greenstein. Lab. of Biochemistry, Natl. Cancer Inst., NIH, Bethesda, Md.

Observation by Neuberger and Sanger (Biochem. J. 37:515, 1943) that rats are capable of nearly normal growth on a lysine-free diet to which ε-acetyl-L-lysine has been added strongly suggests the presence in animal tissues of an enzyme capable of removing the ε-acetyl group. The incubation of various rat tissue homogenates with ε-acetyl-L-lysine followed by treatment of the digests with the specific bacterial L-lysine decarboxylase (Gale and Epps. Biochem. J. 38:232, 1944) has indicated the presence of such activity in kidney, liver, and spleen (in decreasing concentration) and, in barely detectable amounts, in intestine and heart. Purification of the soluble enzyme from fresh rat kidney homogenates was accomplished by means of successive treatment with $Ca_3(PO_4)_2$ gel and ammonium sulfate. A preparation having an activity against ε-acetyl-L-lysine of 15.5 μM/hr/mg N was thus obtained representing about a 100-fold concentration of the enzyme. Purified enzyme which has an optimal activity at pH 7.2 in phosphate buffer is somewhat inhibited, at this pH, by cysteine, glutathione, and ascorbic acid. Preliminary investigations reveal a nearly absolute specificity for various ε-acylated lysine derivatives of the L configuration.

991. EFFECTS OF OXAMIC ACID ON METABOLISM OF ASCITES TUMORS. John Papaconstantinou* and Sidney P. Colowick. McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

Heart muscle lactic dehydrogenase is known to be inhibited by oxamic acid (Glaid, Hakala and Schwert. Abstr. Am. Chem. Soc., Minneapolis, Sept. 1955, p. 18C).

In contrast to inhibitors of any other glycolytic enzymes, a specific inhibitor of lactic dehydrogenase should interfere specifically with cells exhibiting aerobic glycolysis, such as tumors, and not with cells which respire without forming lactic acid. The lactic dehydrogenase activities in extracts of various strains of mouse ascites tumors have been found to be readily inhibited by oxamic acid in the present experiments. The K_i value is about the same as for the muscle enzyme (4×10^{-5} M), and the inhibition is competitive with respect to pyruvate. Anaerobic glycolysis by intact Ehrlich ascites tumor cell suspensions is inhibited 50% by about 8×10^{-3} M oxamic acid and over 90% by 8×10^{-2} M. Aerobic glycolysis is somewhat more sensitive to the inhibitor. The high endogenous respiration of the tumor cells is not appreciably affected by the above concentrations of oxamic acid. The respiration of the cells is inhibited by glucose (Crabtree effect) and is raised back to the endogenous level by concentrations of oxamic acid which suppress aerobic glycolysis. Preliminary tests indicate that mice can tolerate large doses (1 gm/kg) of oxamic acid. Studies on the effects of the drug on tumor growth and metabolism in vivo are therefore contemplated.

992. SULFUR MUSTARD ON GENETIC AND NON-GENETIC FUNCTIONS OF COLI T2 BACTERIOPHAGE. B. Papirmeister,* S. Parsons* and Roger M. Herriott. Chem. Warfare Labs., Army Chem. Ctr. and Johns Hopkins Univ., Baltimore, Md.

It has been found that mustard inactivated T2h phage can be restored by multiplicity or cross-reactivation. Other experiments with isotopically labeled phage showed that a fraction of the inactivated phage was unable to inject its nucleic acid into host cells even though adsorption was normal. Injectibility, host "killing," and ghost lytic action drop at the same rate with increasing dosage of mustard, suggesting a common link among these properties of the protein coat. Cells infected with T2 and then exposed to mustard were also examined. Plaque formation of singly infected cells showed the same sensitivity to the chemical agent as free phage. This sensitivity remained unchanged for the first 8 minutes of the latent period in nutrient broth, after which there was a rapid change to relative resistance. In multiply infected cells the capacity to form plaques was relatively insensitive to mustard from the time of infection. Similarly, cells singly infected with T2h and multiply infected with T2 and then treated with mustard, form T2h although in the absence of T2 they do not. This contrast in sensitivity of singly and multiply infected cells supports the conclusions noted above that multiply infected cells are able, through recombination, to replace mustard inactivated markers by cross or multiplicity substitution processes which lead to the formation of at least one complete phage particle.

993. HYDROLYSIS OF S-ADENOSYLMETHIONINE BY ALKALI. L. W. Parks* and F. Schlenk. Div. of Biological and Med. Research, Argonne Natl. Lab., Lemont, Ill.

Enzyme experiments with S-adenosylmethionine made it desirable to gain information on the stability of this compound and the nature of the hydrolytic products formed from it under various conditions. The preparations used in these studies were extracted from yeast and purified by chromatography. While 5'-methylthioadenosine and homoserine are the main products of hydrolysis by heating in slightly acid or neutral solution, adenine is split off rapidly by alkali at room temperature. No such sensitivity of the adenine ribose linkage to alkali is observed with adenosine or methylthioadenosine; the presence and proximity of the thionium type of linkage in the molecule appears to be responsible for this lability. The fragment remaining after the removal of adenine behaves like a thionium compound in chromatography; it is devoid of ultraviolet absorption, but it responds to the orcinol and ninhydrin reagents. Amino acid oxidases do not react with this substance. Heating of the alkaline solution of this compound causes rapid destruction of the pentose and formation of methionine and small amounts of homoserine. It is believed that the main reaction sequence may be represented as follows: S-adenosylmethionine ⟶ adenine + S-ribosylmethionine; S-ribosylmethionine ⟶ methionine + pentose fragments. Methionine is readily determined by the conventional tests. S-ribosylmethionine is hydrolyzed exclusively to homoserine and thiomethylribose with acid treatment. S-adenosylethionine, also obtained from yeast, resembles S-adenosylmethionine closely in its chemistry. The relationship of the chemical degradation of S-adenosylmethionine to the enzymatic formation of methionine from the thionium compound will be discussed. (Work performed under the auspices of the Atomic Energy Comm.)

994. NATURE OF LEUCINE AMINOPEPTIDASE FROM AN ASCITES TUMOR. Elizabeth K. Patterson (intr. by Mary A. Bennett). Inst. for Cancer Research and Lankenau Hosp. Research Inst., Philadelphia, Pa.

Leucine aminopeptidase from hog kidney has been purified previously by two methods yielding in one case (Spackman, Smith and Brown. J. Biol. Chem. 212:255, 1955) a typical protein, and in the other (Binkley, Olsen and Torres. Abstr. Am. Chem. Soc., Minneapolis, Sept. 1955, p. 36C) a complex containing nucleoside diphosphates, glucosamine and a simple peptide. The latter purification procedure has been applied to the supernatant fraction from homogenates of Lettre Ehrlich mouse ascites carcinoma cells. After prolonged digestion with proteolytic enzymes (trypsin, chymotrypsin, carboxypeptidase) and salt-alcohol precipitation (M NaCl or 0.5 M $Ba(Ac)_2$), nucleoprotein-like complexes were obtained in which the protein concentration was reduced 200-fold with little loss in enzyme activity. Further successive digestions with proteolytic enzymes, ribonuclease, snake venom diesterase, and alkaline phosphates followed by reprecipitations have led to preparations (yield 10-40%) of C_1 50-70, values not far from that (C_1 88) obtained by Smith et al. In addition to TCA and bromsulfalein precipitable protein, the ascites enzyme preparation contains glucosamine and nucleoside mono- and di-phosphates with guanine apparently the predominant base. This composition is similar to that of the aminopeptidases purified by a similar method by Binkley et al. with the exception of the size of the polypeptide component. It should be noted, however, that in the most purified ascites fractions, leucine aminopeptidase activity is directly proportional to protein (bromsulfalein precipitable) concentration and shows no discernable quantitative relationship to other substances present. Further attempts are in progress to evaluate the function of the nonprotein fragment.

995. CRYSTALLIZATION AND PHYSICAL PROPERTIES OF BOVINE LIVER ALDOLASE. Robert J. Peanasky* and Henry A. Lardy. Dept. of Biochemistry and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

The enzyme which catalyzes the reaction hexose diphosphate ⟷ dihydroxy acetone phosphate + glyceraldehyde phosphate has been crystallized from beef liver. Purification was accomplished by: 1) extraction of ground beef liver in 0.15 M KCl at pH 7.6; 2) fractionation at -16°C in the presence of 0.5 M KCl (initial concentration) between 48 and 61% (v/v) with methanol; 3) heat denaturation of inert proteins at 40°C for 75 min in the presence of 0.3 M KCl (initial concentration) and 10% (v/v) methanol at pH 9.6; 4) removal of more inert protein from the supernatant fraction of step 3 with alumina gel; 5) adsorption of the protein at pH 9.6 on alumina gel and elution with 0.4 saturated ammonium sulfate pH 7.4; 6) precipitation with ammonium sulfate between 0.4 and 0.7 saturation at pH 5.4. 7) This precipitate when suspended in 0.4 saturated ammonium sulfate at 0°C and pH 7.4 crystallized immediately. Recrystallization can be carried out at 0.39 saturation with ammonium sulfate pH 7.4 and 0° giving rise to long needle shaped crystals. The preparation represents a 100-120 fold purification in 20 - 27% yield. It migrates as a single component in electrophoresis in 0.1 ionic strength phosphate buffers between pH 6 and 8 and is isoelectric between pH 6.6 and 6.7. The molecular weight is 160,000 calculated from S_{250}, 8.49×10^{-13} sec^{-1}; D_{250}, 5.18×10^{-7} cm^2/sec, and a partial specific volume $_{250}$, 0.74_3 (all determined in 0.15 M KCl, 0.01 M phosphate buffer pH 6.7). A comparison of the physical properties and substrate specificity of this enzyme and of rabbit muscle aldolase will be presented.

996. INFLUENCE OF SPECIFIC FATTY ACIDS ON THE DEVELOPMENT OF ATHEROSCLEROSIS IN MINIATURE PIGS. James J. Peifer* and Walter O. Lundberg. Hormel Inst., Univ. of Minnesota, Austin.

Fatty acid esters of cholesterol have been found to accumulate in sclerotic tissues. Saturated fats have been reported to elevate plasma cholesterol while more unsaturated fats bring about a decrease in blood cholesterol in hypercholesteremic patients. Hammond and Lundberg (Arch. Biochem. 57:517, 1955) and Peifer and Holman (Arch. Biochem. 57:520, 1955) have demonstrated a relationship between EFA, cholesterol and atherosclerosis. Further studies have been carried out in miniature pigs using specific fatty acids. Eight-week-old miniature pigs were placed on a fat-free diet containing 0.1% cholesterol for 2 months. At the end of this period ethyl esters of saturated (75% palmitate and 25% myristate and laurate), oleic, linoleic, and linolenic (containing some 25% linoleate) acids were substituted for 10% of the dietary dextrose and fed individually to four different groups of pigs. Dietary cholesterol was increased to 0.5% at this time. Following 1 month on the fat diet the animals were killed. Aortas from pigs receiving ethyl linolenate showed only a small amount of plaque formation. Pigs receiving ethyl linoleate had more extensive aortic deposits, while those receiving oleate or saturated esters had very extensive deposits over the entire surface of the thoracic aorta. Results from this experiment suggest that the degree of unsaturation of dietary fatty acids plays a predominant role in the development of atherosclerosis. Studies on the fatty acid distribution in tissue lipids will also be reported.

997. MICROSOMAL DPNH CYTOCHROME c REDUCTASE. Nathar Penn* and Bruce Mackler. Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

DPNH cytochrome c reductase associated with preparations of crude beef liver microsomes has been isolated and purified as a particulate entity. The purified enzyme catalyzed the oxidation of DPNH but not of succinate or TPNH with cytochrome c or ferricyanide as electron acceptors, and was insensitive to high concentrations of antimycin A and amytal. Purified preparations were 10 times as active as crude microsomes in the oxidation of DPNH by cytochrome c, but were only 2 - 3 times as active when ferricyanide was used as acceptor. This suggests the presence of an additional diaphorase in the crude microsomal preparations. Purified preparations contained flavin, cytochrome b and nonheme iron in the molecular ratio of 1 : 2 : 8. The lipid content (percentage of total dry weight) was 65%. Only small amounts of RNA were present. The concentration of cytochrome c reductase activity and cytochrome b ran parallel. The flavin was not extractable with trichloracetic acid, nor was the falvoprotein detached from the enzyme by treatment with acid acetone. The preparation differs in these respects from various particulate beef heart preparations (DPNH oxidase, ETP) which catalyze the oxidation of DPNH.

998. DEGRADATION OF RIBONUCLEOPROTEIN FROM RAT LIVER. Mary L. Petermann and Mary G. Hamilton.* Sloan-Kettering Inst. for Cancer Research, New York City.

Ribonucleoprotein which has been prepared from rat liver (J. Biol. Chem. In press) plays a fundamental role in protein synthesis (Littlefield, Keller, Gross and Zamecnik. J. Biol. Chem. 217:111, 1955). In an effort to elucidate the structure of the nucleoprotein particle, its degradation has now been undertaken. Since the intact nucleoprotein gives characteristic ultracentrifugal and electrophoretic patterns, these techniques can be used to follow the changes produced by the various agents studied. Under very mild treatment, such as exposure to 0.2 M sodium chloride, the sedimentation rate of the principal nucleoprotein boundary, B, is reduced from 78 S to about 47 S. A similar change takes place in potassium phosphate of ionic strength 0.10 at pH 7.7. On electrophoretic analysis in the phosphate buffer, although the boundaries show considerable spreading, only a trace of free ribonucleic acid (RNA) is seen. On prolonged dialysis against 0.01 M versene plus 0.09 M sodium chloride at pH 8.3, or against glycine buffer at pH 10.8, the nucleoprotein is broken down into much smaller fragments, with sedimentation rates as low as 3 S. On electrophoretic analysis these degradation products give complex patterns which differ markedly in the two limbs of the cell; the leading boundary in the ascending limb has no counterpart in the decending limb. Material withdrawn from this boundary appears from its ultraviolet absorption curve to consist almost entirely of free RNA. Thus under these conditions a partial dissociation of RNA and protein is achieved.

999. PAPER ELECTROPHORESIS OF SERUM PROTEINS IN CONTROL AND DIABETIC RATS. Ruth D. Peterson and Clarissa H. Beatty. Biochemistry Dept., Univ. of Oregon Med. School, Portland.

Prior to studying the effect of diet and hormones on serum proteins we have compared fractions separated by paper electrophoresis (Spinco Model R, Veronal pH 8.6, μ 0.075, 10 ma, 17 hr, Spinco analytrol) in control, depancreatized and alloxanized female rats. Twelve fed alloxan diabetic rats showed a decrease, 13.1% in serum albumin compared to 17 fed controls ($P < .005$), an increase in β-globulin, 7.9% ($P < .005$) and no change in % α_1 or α_2-globulins. Five 20-hr fasted alloxan

diabetics showed elevated β-fractions. Total serum proteins were lower in alloxan diabetics (-0.95 g% P .005) and there was an increase of 0.29 g% (P< .005) in β-globulin, no change in α -globulin and a decrease in α -globulin (P < .005). Seven pancreatectomized rats with high fasting blood sugars showed a decrease of 8% in serum albumin (P < .01), a decrease of 2.8% in α_2-globulin (P < .05) and an increase of 6.7% in β-globulin (P < .005) compared with 17 controls. There was no change in total serum protein following pancreatectomy (P > .10). Six rats, deprived of pancreatic exocrine secretion, showed no change in % serum albumin, α_1 or β-globulins or total serum protein, and a decrease of 2.4% in α -globulin (P < .05). Changes in % albumin and β-globulin are similar to those reported in dianetic humans and do not agree with the changes reported in male alloxan diabetic rats (Nichols et al. Diabetes 5:468, 1956). (Aided by PHS grants A-213 and A-1157, and the Glidden Co.)

1000. DIFFERENCES BETWEEN NORMAL AND ARTHRITIC SYNOVIAL FLUID AS INDICATED BY "SIALIC ACID" REAGENTS. Ward Pigman, William L. Hawkins,* David Platt,* Howard L. Holley* and Mary Grace Blair. Arthritis and Rheumatism Research Lab., Univ. of Alabama Med. Ctr., Birmingham.

A substance (or substances), which occurs in some fractions of blood serum, produces a purple color when reacted in strongly acid solution with diphenylamine or with p-dimethylaminobenzaldehyde (Ehrlich reagent; see Werner et al. Acta Soc. med. upsalien. 57:230, 1952). The quantity of the color producer is increased in the serums of persons afflicted with malignant or inflammatory diseases. Diphenylamine has been explored as to its possible value in assessing the efficacy of drugs in the treatment of rheumatic fever (Coburn et al. J. Chronic Diseases 3:140, 1956) and of rheumatoid arthritis (Fearnley et al. Ann. Rheumat. Dis. 14:226, 1955 and Cecchi et al. Ibid. p. 267). The present paper extends this type of investigation to the examination of arthritic synovial fluid. A correlation is found between decreased optical density with each of the two most common "sialic acid" reagents and improved clincial state after treatment. A test of randomly selected post-mortem fluids suggests that the amounts of the color producer are much less in normal fluids than in arthritic fluids and fluids of persons with certain other diseases. In the main, the two reagents appear to measure the same substance. The diphenylamine reagent is more sensitive but less selective than the Ehrlich reagent.

1001. A SOLUBLE ENZYME CATALYZING AN EXCHANGE BETWEEN P_i^{32} AND ATP. G. W. E. Plaut. Dept. of Biochemistry, New York Univ. College of Medicine, New York City.

Studies of Boyer et al. and Swanson have demonstrated an exchange of labeled inorganic phosphate (P_i^{32}) with the terminal phosphate of ATP with intact mitochondria in the absence of substrate oxidation. Evidence has been presented which suggests a relationship between this reaction(s) and the process of oxidative phosphorylation. For these reasons it was of interest to determine if an exchange reaction between P_i^{32} and ATP could be demonstrated with soluble preparations from mitochondria. Aqueous extracts from rat liver (or heart) mitochondria prepared according to Lardy and Wellman led to an incorporation of P_i^{32} into ADP and ATP which required Mn^{++} for activity. The enzyme was purified 15-20 fold from aqueous extracts of pig liver particles. This preparation was virtually free of myokinase, ATP-ase, and IDP-ase activity; it exchanged about 0.7 μM of the terminal P of ATP in 30 min at 25°/mg protein. Although the exchange involved the terminal P of ATP, the presence of ADP was required for maximal activity. ITP, GTP, UTP, or CTP did not replade ATP; and IDP, UDP, GDP, or CDP did not replace ADP. The activity of purified enzyme was neither diminished by exhaustive dialysis nor stimulated by the addition of a variety of materials. The concentrations of ADP and ATP were virtually unchanged during the course of the reaction. It would appear that the exchange might involve the following types of reactions: 1a) $ATP + X \rightleftharpoons ADP + XP$, 1b) $XP + Y \rightleftharpoons XY + P$ (X and Y being moieties associated with the enzyme) or 2) $ATP + X \rightleftharpoons ADP\text{-}X + P_i$. Although the reaction occurs in mitochondrial extracts it is not certain if it is a "partial reaction" of the P_i^{32}-ATP exchange observed with intact mitochondria since it is insensitive to dinitrophenol or azide. It is also insensitive to iodoacetate but is inhibited by pCMB and arsenate.

1002. ENZYMATIC SYNTHESIS OF GLUCOSAMINE-6-PHOSPHATE IN RAT LIVER. B. M. Pogell and R. M. Gryder (intr. by E. K. Marshall, Jr.). Wilmer Inst., Johns Hopkins Med. School, Baltimore, Md.

A soluble enzyme system which forms D-glucosamine-6-phosphate and L-glutamate from D-glucose-6-phosphate (G6P) and L-glutamine has been partially purified from rat liver extracts. L-asparagine, NH_4^+, glutamate, and NH_4^+ plus glutamate were inactive as sources of the amino group. Fructose-6-phosphate (F6P) could replace G6P in the crude extract, but the ratio of activity with F6P compared to G6P decreased during purification. The hexosamine phosphate was identified before and after hydrolysis with wheat germ acid phosphatase by paper chromatography and the Blix-Elson-Morgan and Dische-Borenfreund color reactions. Glutamate was identified by paper chromatography as the only new amino acid formed by this enzyme, and only appeared when both G6P and glutamine were present as substrates. A great hindrance in the study of this enzyme has been its instability. One of the substrates, G6P, was found to exert a pronounced stabilizing effect. In a dialyzed ammonium sulfate precipitated fraction, most of the activity disappeared after 20 min preincubation at 38°, whereas with G6P present, all activity was retained for at least 2 hr. That concentration of G6P which gave maximal enzyme activity (10 μM/ml) also provided maximal protection during preincubation. When G6P was included in the isotonic KCl used for homogenization, aminotransferase activities of 1.7 - 4.4 times that obtained with no substrate present were found in the extracts, whereas addition of F6P, glucose, sucrose or β-glycerophosphate produced little or no effect.

1003. ACTION OF CLOSTRIDIUM PERFRINGENS ON OROSOMUCOID. Edwin A. Popenoe and Ruth M. Drew.* Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Action of an enzyme present in filtrates of Clostridium perfringens on orosomucoid, an α_1-glycoprotein of normal human plasma, has been examined. For this study orosomucoid was isolated from nephrotic urine. The strain of Cl. perfringens (A.T.C.C. 10543, type not known) was grown anaerobically in Todd-Hewitt medium for 18 hr. The organisms were removed by centrifugation followed by filtration. The filtrate could be used directly as a source of enzyme activity, or the enzyme could be concentrated by ammonium sulfate precipitation. When orosomucoid was incubated with such filtrates,

sialic acid became freely dialyzable. No proteolysis could be observed. Neither was there any significant change in viscosity. The enzymatic reaction proceeded with maximum velocity at pH 5.0 - 5.5 in acetate buffer. No requirement for metallic ion activators could be demonstrated. The isoelectric point of orosomucoid is pH 2.7 (Schmid) but by removal of sialic acid, the isoelectric point was raised to approximately pH 5.0 in sodium acetate buffer, ionic strength 0.1. A comparison of the titration curves of orosomucoid and the sialic acid-free mucoid showed a loss of 16 - 17 strongly acidic groups per protein molecule (assumed molecular weight 45,000) during the enzymatic reaction. The basic portions of the two curves were parallel as far as pH 11, indicating that the enzymatic reaction produced no change in the number of basic groups. Dye titrations at pH 11.5 (method of Fraenkel-Conrat and Cooper) indicated a loss of approximately 30 acid groups per mole of protein as a result of the enzymatic reaction. A second enzyme found in some Cl. perfringens filtrates destroys sialic acid. This enzyme shows a maximum activity between pH 6 and pH 8. It is probably similar to the enzyme recently observed by Heimer and Meyer in filtrates of Vibrio cholerae. (Supported by the Atomic Energy Comm.)

1004. PRODUCTS OF FATTY ACID SYNTHESIS BY A SOLUBLE PIGEON LIVER SYSTEM. John W. Porter and R. W. Long.* Inst. for Enzyme Research, Univ. of Wisconsin, and VA Hosp., Madison.

Earlier abstracts (Wakil, Porter and Tietz. Fed. Proc. 15:377, 1956; Gibson and Jacob. Fed. Proc. 15:261, 1956) have reported cofactor requirements, fractionation of enzymes and demonstration of possible reactions of fatty acid synthesis. Further work on intermediates and end products of the reaction has shown that acetyl CoA is an intermediate in fatty acid synthesis. Fractionation of the fatty acids synthesized has shown that palmitic (60-80% of total), myristic (15-30% of total) and lauric (5-12% of total) acids are the products. Further, 80-95% of the total acids are present as free (unesterified) acids. The esterified acids are extractable by the Kornberg, Pricer procedure (J. Biol. Chem. 204:329, 1953). On chromatography the esterified acids behave as palmityl CoA. Enzymatic assay with fatty acyl CoA dehydrogenase shows the ester to be a long-chain acyl CoA compound. Saponification, extraction and chromatography show the esterified acids to be palmitic and myristic acids. The long-chain fatty acyl CoA's could have arisen either directly by way of the synthesis mechanism or from the free fatty acids in presence of the long-chain thiokinase, ATP and CoA. This point is currently under investigation.

1005. SOME PROPERTIES OF RIBONUCLEIC ACIDS. Joseph L. Potter* and Alexander L. Dounce. Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The presence of contaminating unbound protein in RNA samples containing firmly bound amino acids has been fairly well excluded by the observation that added protein (serum albumin, chymotrypsin, or insulin) can be completely removed from the RNA by treatment with detergent or by the use of Dowex-1 anion exchanger columns. Extended exposure of the RNA samples to detergents always fails however to remove the bound amino acids or to raise the percentage of phosphorus to the theoretical value. If solutions of whole RNA are passed through Dowex-1 columns at pH 8.5, the RNA is retained by the resin. When 1 N HCl is used for elution after extensive washing of the resin with water and dilute acid to remove protein, the eluted RNA still contains bound amino acids. The amino acids bound to RNA are only those known generally to be present in the free state in the tissue of origin. If RNA binds amino acids prior to peptide chain synthesis, the free amino acids of the cell should be the first ones accumulated. Walker tumor RNA, which contained whole protein or peptide chains of some length, still showed the same amino acid pattern as that of liver or pancreas RNA. The composition of RNA-ase digests of calf pancreas RNA differed from that reported in the literature for yeast and calf liver RNA. There were bound amino acids in the polynucleotide fractions which might have prevented the action of RNA-ase on sites that otherwise would be susceptible to this enzyme.

1006. ISOLATION FROM THYMUS OF A NUCLEOTIDE CONTAINING DEOXYCYTIDYLIC ACID. Richard L. Potter and Vina Buettner-Janusch (intr. by Lawrence H. Louis). Atomic Energy Commission Lab. and the Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.

Gradient elution of thymus extracts from anion-exchange columns has previously (Potter and Schlesinger. J. Am. Chem. Soc. 77:6714, 1955) yielded the mono-, di- and triphosphates of deoxycytidine (D-CMP, D-CDP, D-CTP). Under the same conditions several unidentified nucleotides containing 2 atoms of P per mole of cytosine were eluted before CMP and D-CMP. On the basis of their relatively low affinity for the resin they were presumed to be substituted cytidine diphosphate compounds (CDPX). Rechromatography with ammonium formate at pH 9.3 resolves this mixture into 2 fractions (I, II). Paper chromatography (isobutyric acid-ammonia) of I yielded 2 fractions (IA, IB) each of which contained 2 atoms of P per mole of cytosine and which appeared to be identical except for their cytidylic acid moiety. Boiling IA in N HCl for 15 min liberated little or no inorganic phosphate but resulted in an 80 - 85% conversion to equimolar quantities of D-CMP and an unidentified phosphorus compound (XP). Acid hydrolysis of IB gave CMP and a phosphorus compound which seemed to be identical to XP. The acid lability of IA and IB is interpreted as indicating a pyrophosphate bond. The following structure for IA is proposed: cytosine-deoxyribose-P-P-X (D-CDPX). Anion-exchange chromatography with formic acid revealed the elution order to be XP, D-CDPX and D-CMP suggesting that X is basic relative to deoxycytidine.

1007. EFFECT OF CHOLESTEROL FEEDING ON THE PLASMA LIPIDE FRACTIONS OF ADRENALECTOMIZED DOGS. B. S. Powers and N. R. Di Luzio (intr. by D. B. Morrison). Div. of Physiology, Univ. of Tennessee, Memphis.

Previous studies have demonstrated that adrenalectomized dogs maintained on desoxycorticosterone acetate show a progressive decrease in plasma phosphatide, cholesterol, and lipoprotein concentrations. To extend these observations to factors which might modify this response, the effect of cholesterol feeding on the plasma lipide alterations of these dogs was studied. Eleven dogs were bilaterally adrenalectomized in a single stage operation and subsequently maintained on Purina chow and DCA for 3 or 4 wk. A cholesterol supplement of 1 gm/kg/day was fed to 5 of these dogs. Plasma samples obtained prior to, and at weekly intervals after adrenalectomy were analyzed for phosphatide, cholesterol and total lipides. In agreement with our previous observations, adrenalectomy was associated with a

mean decrease in phosphatide, cholesterol, and neutral fat of 68, 51, and 71%, respectively. In marked contrast, the cholesterol-fed dogs had phosphatide and cholesterol levels which were 25 and 60% respectively above the preoperative values. The termination of daily cholesterol feeding was associated with a prompt and marked decrease in all of the plasma lipide fractions. Thus, an amount of cholesterol, which did not produce any lipide alterations in normal dogs, was not only able to prevent the characteristic decline in all of the plasma lipide fractions after adrenalectomy, but also resulted in an elevation of the plasma lipide levels. The mechanism whereby cholesterol prevents this depression is currently under investigation. (Supported in part by PHS grant H2320 and Memphis Heart Assoc.)

1008. IN SITU INTERCONVERSIONS OF INTRAMITOCHONDRIAL NUCLEOTIDES. Berton C. Pressman and Jeanne Lange (intr. by Laurens Anderson). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

Incubation of rat liver mitochondria in isotonic sucrose, 0.015 M in ferricyanide, for 5 min at 0°, leads to the phosphorylation of about 70% of the intramitochondrial ADP to ATP with a corresponding disappearance of intramitochondrial inorganic phosphate, as shown by chromatography on Dowex 1 formate of the perchloric acid extractable nucleotides. The AMP level remains unchanged indicating the lack of adenylate kinase equilibria under these conditions but there is a simultaneous conversion of the bands referred to by Potter and coworkers as "AD" and "ADX" to DPN and TPN. This, together with data obtained independently by Brum and Potter, led to the identification of AD and ADX as acid decomposition products of DPNH and TPNH respectively and implies that the pyridine nucleotides in situ are predominantly reduced. If, alternatively, mitochondria are dissolved in the nonionic reagent 45% urea - 4% triton, and the resulting solution passed directly through the chromatographic column, the major portion of the pyridine nucleotides are found in the oxidized state. Since this treatment neither oxidizes added reduced nucleotides nor reduces added oxidized nucleotides, the acid and triton-urea digestion procedures lead to conflicting pictures of the redox state of mitochondrial nucleotides as yet unresolved. Although the urea-triton reagent is powerful enough to dissolve whole yeast and bacteria cells, its neutrality enables such acid-labile substances as reduced pyridine nucleotides to be chromatographed directly from tissue without decomposition. Other conditions producing redox and phosphorylative conversions of mitochondrial nucleotides will be discussed.

1009. FREE AMINO GROUPS OF BENCE-JONES PROTEINS. Frank W. Putnam. Dept. of Biochemistry, College of Medicine, Univ. of Florida, Gainesville.

Physicochemical characterization and amino end-group analysis have demonstrated that the apparently homogeneous Bence-Jones (BJ) proteins produced by different patients with multiple myeloma are not identical. Indeed, some of these unique urinary proteins appear to differ in chemical structure and to be characteristic of the individual (F. W. Putnam and A. Miyake. Science 120:848, 1954). Extension of this work to a total of 14 specimens together with collaborative study of their antigenic behavior by Dr. Leonhard Korngold (Sloan-Kettering Inst.) has permitted classification into 2 major groups, antigenic types A and B. All proteins of type A are devoid of N-terminal aspartic acid and have an $s_{20,w}$ of 3.4 S, but the 8 specimens could be differentiated by other properties, e.g., pI, electrophoretic mobility, crystallizability, or amino end-group. No N-terminal group was detected in 5 specimens. Type B proteins all had N-terminal aspartic acid but differed strikingly in physical constants and were somewhat less homogenous. Variation in the number of epsilon-amino groups did not correlate with antigenic type. There is no apparent relationship of serum electrophoretic pattern with antigenic type or chemical or physical properties of the BJ proteins. Experiments on the rate of excretion of labeled BJ protein in a patient and in rabbits together with study of the recovered protein suggest that the differences in BJ proteins are characteristic (possibly owing to a metabolic block) rather than resulting from secondary degradative changes during renal excretion.

1010. CONGENITAL HYPOPROTHROMBINEMIA VERA STUDIED BY THE ONE- AND TWO-STAGE PROTHROMBIN PROCEDURES. Armand J. Quick and Clara V. Hussey.* Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

In acquired hypoprothrombinemic states, a decrease of both prothrombin and stable factor (Factor VII) occurs. In the rare congenital type of hypoprothombinemia, prothrombin alone is decreased. In 2 brothers with this condition, the prothrombin time has remained 19-26 sec for the past 10 yr. On the basis of the normal prothrombin time of 12 sec, the calculated prothrombin activity is about 30% of normal. The total prothrombin time is 16-19 sec while that of normals is 8-9 sec. The calculated total prothrombin of these boys averages 10% of normal. The prothrombin time is also prolonged with Russell viper venom and with a mixture of Russell viper venom and rabbit brain extract. The prothrombin time is not corrected by adding stable factor prepared by the method of Koller, nor by an excess of labile factor (Factor V), but on mixing their fresh oxalated plasmas with an equal volume of normal plasma, a value of 12 sec is obtained. By means of the 2-stage method as modified by Ware and Seegers, a prothrombin of 31 units is found which is 10-11% of normal. It may be concluded that in congenital hypoprothrombinemia vera both prothrombin and prothrombinogen are decreased, but that the other factors of the prothrombin complex are normal. (Aided by Research Grant (H 1612 C-10) from the Public Health Service.)

1011. TRANSFORMATION OF RIBONUCLEOPROTEIN IN AN ACELLULAR PREPARATION FROM RABBIT RETICULOCYTES. M. Rabinovitz and M. E. Olson.* Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

Ribonucleoprotein of the microsome fraction of rabbit reticulocytes attains an initial high level of incorporated radioactivity when the cells are incubated with tracer levels of radioactive leucine. The activity reaches a maximum in 2 min and then drops precipitously with the concurrent transfer of leucine to the soluble protein, hemoglobin (Exper. Cell Research 10: 747 (1956)). This transfer of radioactivity from the particulate ribonucleoprotein to the soluble protein could be obtained with cell-free preparations of microsomes isolated from reticulocytes which had been previously incubated with radioactive leucine. The reaction was found to require the presence of the soluble fraction of reticulocytes and phosphoglycerate, and was stimulated by ATP and Mg^{++}. The transfer was essentially complete after a 5 min incubation at

37.5°C and was not diminished in the presence of nonradioactive leucine. Carbonmonoxyhemoglobin (COHb) was isolated by chromatography of the carbon monoxide treated soluble proteins on a carboxylic acid resin (IRC 50 XE 97) equilibrated with 0.2 M sodium phosphate buffer, pH 6.5. The COHb isolated from the fortified incubation mixture had a specific activity which was 4 times greater than that of the COHb isolated from the unfortified preparation. In addition, a red protein fraction preceding the major COHb band was observed only in chromatograms of the soluble proteins from fortified incubations. The specific activity of this protein was higher than that of the COHb. The results support the postulate that part of the protein of reticulocyte ribonucleoprotein is a precursor of globin. However, a balance of recovered radioactivity indicated that in addition to the COHb and red protein fore-band some labeled noncolored proteins were also released from the ribonucleoprotein in these cell-free preparations.

1012. FORMATION, ISOLATION AND PROPERTIES OF 5-FORMIMINOTETRAHYDROFOLIC ACID. Jesse C. Rabinowitz and W. E. Pricer, Jr.* Natl. Insts. of Health, Bethesda, Md.

A formimino transferase catalyzes the reaction: formiminoglycine + tetrahydrofolic acid $\rightleftharpoons$ 5-formiminotetrahydrofolic acid + glycine (J. Am. Chem. Soc. 78:5702, 1956). This enzyme has been purified 10-fold from lyophilized Clostridium cylindrosporum by precipitation at pH 5 and absorption of impurities on calcium phosphate and alumina C_γ. The K_s values for dl-THF and FIG are 2.8×10^{-3} and 11.1×10^{-3} respectively. To isolate the 5-formimino-THF formed, 4.5 mM of dl-THF, 245 mM of formiminoglycine, 3.5 mg of enzyme and other additions were incubated to completion of the reaction (25°, 45 min). The mixture was chromatographed on Dowex-1-acetate at 4° using gradient elution with 0.2 N acetic acid. 54% of the product was recovered in 1860 ml of eluant. 250 mg of a pale yellow solid was isolated by lyophilization. At pH 7.0, 5-formimino-THF shows absorption maxima at 222 and 285 mμ, as does 5-formyl-THF. In the presence of a second purified enzyme, cyclodeaminase, 5-formimino-THF, but not 5-formyl-THF, is converted to 5,10-methenyl-THF (anhydrocitrovorum factor, anhydroleucovorin) with the simultaneous formation of an equivalent amount of ammonia. The 5,10-methenyl-THF formed is a single diastereoisomer since it is completely converted to 10-formyl-THF by the enzyme cyclohydrolase, whereas only 50% of a synthetic sample of dl-5,10-methenyl-THF undergoes this reaction. These results demonstrate that the 5-formimino-THF isolated is also a single diastereoisomer. 5-Formimino-THF, in distinction to both 5-formyl-THF and 5,10-methenyl-THF, shows a fluorescence spectrum with an emission maximum at 460 mμ when activated at 285 mμ. 5-Formimino-THF shows oxygen stability characteristic of 5-substituted tetrahydropteridines. The compound is relatively stable at 0°. However, over the pH range of 5--9, its half life at 37° is 1 hr and at 100° it is less than 1 min under aerobic or anaerobic conditions. When sterilized by filtration, 5-formimino-THF is twice as active as dl-5-formyl-THF in supporting growth of Pediococcus cerevisiae.

1013. PHOSPHATE TRANSFER TO YOLK PHOSPHOPROTEIN. Murray Rabinowitz (intr. by M. E. Jones). Biochemical Research Lab., Massachusetts General Hosp., Boston.

Protein-bound phosphate has attracted much attention recently. Noteworthy are the phosphate transfer to casein from ATP by liver acetone powder extracts (Burnett and Kennedy. J. Biol. Chem. 211: 969, 1954) and the isolation of serine phosphate from liver and other phosphoproteins (Kennedy; Aagren). We now wish to report experiments on phosphate transfer from ATP^{32} to the egg yolk protein phosphovitin, which appears to contain phosphate uniformly bound to serine (Lipmann and Levene. J. Biol. Chem. 98:109, 1932). Acetone powder extracts of various organs were used, the most active being that obtained from pigeon brain. Liver extracts, including those of laying chickens, were less active. Phosphovitin, prepared according to Mecham and Olcott was used either as such or after partial dephosphorylation with Sundararajan's spleen protein phosphatase. Optimal activity was shown after 15-25% dephosphorylation. Dephosphorylated preparations were 2 - 3 times as active as untreated phosphovitin; in contrast partial dephosphorylation by alkali inhibited. Radioactive phosphovitin was isolated and its P^{32} characterized as protein bound by its alkali lability and acid stability. The enzyme from pigeon brain is now being purified and the reversibility of the reaction between ATP and phosphovitin is being investigated.

1014. CHEMICAL AND EEG CHANGES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING 700r TOTAL BODY X-IRRADIATION. E. S. Redgate,* W. O. Caster and W. D. Armstrong. Depts. of Physiology and Physiological Chemistry, Univ. of Minnesota, Minneapolis.

Three hundred and fifty gram male white rats given 700 r total body X-irradiation show a 10-15% decrease ($P < .05$) in brain DNA content at 2 definite periods: 12 hr and 4-6 days post X-ray. There was a decrease in brain water ($P < .01$) and sodium ($P < .05$) at 1 day and an increase in brain water ($P < .05$) and chloride ($P < .01$) at 3 days. Electrocorticograms, representing potential differences between the left and right hemispheres, were recorded on each of a group of 8 rats. Simultaneously, the Offner wave analyzer provided a Fourier analysis of the brain potentials at each of 24 frequencies between 1.5 and 30 cycles/sec. In the 1.5-2.0 cycles/sec range, the mean amplitude diminished to approximately 80% of the control level within 3 hr following total body irradiation, and remained low ($P < .05$) for the remainder of the experimental period. At the 3.5-4 cycles/sec range there were 2 distinct periods of decreased ($P < .01$) amplitude which were centered at 3 hr and 6-10 days post X-ray. At 11 and 12 cycles/sec there were no detectable changes. At all other frequency ranges the means show minima at 3-12 hr and 6-8 days but these changes were not statistically significant. These 2 periods of decreased brain DNA concentration and selective reductions in electrocorticographic potentials occurred just prior to the acute and LD_{50} death periods, respectively.

1015. LIPOIC ACID-ACTIVATING ENZYME SYSTEM. Lester J. Reed, Franklin R. Leach,* M. Koike* and Mark E. Levitch.* Clayton Fndn. for Research, Biochemical Inst., and Dept. of Chemistry, Univ. of Texas, Austin.

Extracts of lipoic acid-deficient Streptococcus faecalis, strain 10C1, can catalyze a dismutation of pyruvate and α-ketobutyrate provided the extracts are first incubated with lipoic acid (Biochim. et biophys. acta 18:297, 1955). During the incubation lipoic acid is converted to an enzymatically active, nondialyzable form. Fractionation of the lipoic acid-deficient extracts with protamine yielded a protamine precipitate fraction, which contained the pyruvate (and α-ketobutyrate) oxidation system, and a supernatant fraction, which contained a lipoic acid-activating

enzyme system. Both enzyme fractions had to be incubated simultaneously with lipoic acid to obtain an active dismutation system. When more highly purified enzyme fractions were employed, it was necessary to add ATP to the incubation mixture. Lipoic acid and ATP could be replaced by synthetic lipoyl adenylate. A lipoic acid-activating enzyme system was obtained also from extracts of Escherichia coli, Crookes strain. Furthermore, a purified pyruvate oxidation system from E. coli, which contained firmly bound lipoic acid, was inactivated by treatment with an enzyme fraction from S. faecalis, which catalyzes the hydrolysis of amides and esters of lipoic acid. Pyruvate oxidation activity was restored by incubating the inactive preparation simultaneously with lipoic acid and ATP (or synthetic lipoyl adenylate) and the lipoic acid-activating enzyme system from E. coli. It appears that the activating system participates in both the activation of lipoic acid through its carboxyl group and the subsequent incorporation of lipoic acid into the pyruvate oxidation system.

1016. TRANSPORT OF GLUCOSE AND OTHER SUGARS ACROSS THE CELL MEMBRANE OF THE HUMAN ERYTHROCYTE. D. Reinwein,* C. F. Kalman* and C. R. Park. Dept. of Physiology, Vanderbilt Univ., Nashville, Tenn.

Using improved optical and chemical methods, the initial transport rate of glucose and other sugars across the cell membrane was measured as a function of concentration. Saturation curves were obtained with D-glucose, 5 other aldohexoses and D-xylose. Saturation was not observed with 6 other aldopentoses and 2 ketohexoses at concentrations as high as 0.09 M. Inhibition of transport of all the above sugars by glucose (competitive) and mercurials was noted, but the transport of D-glucosamine, DL-glyceraldehyde and D-erythrose was not affected. Phloretin inhibited both the mercury sensitive and insensitive systems. The C-1 alcohol and acid analogues of glucose and several C-1 glucoside derivatives were not measurably transported. The glucose transport Q_{10} was 2.7. 0.01 M fluoride did not inhibit transport although it completely prevented glucose phosphorylation. These observations, which confirm and extend those of LeFevre, Wilbrandt and Widdas, indicate that monosaccharide transport involves a reversible combination of the sugar with a specific membrane constituent. β-D-glucose was found to have the highest affinity for this combining site. The "K_m" for equilibrium glucose was about 5×10^{-3} M at pH 7.5. Mutarotase activity was not found in whole cells or membrane preparations. Glucose transport in adult rabbit erythrocytes could also be described by Michaelis-Menten kinetics with a slightly lower "K_m" value and a transport maximum 1000x smaller. Glucose and fructose did not compete in the rabbit system and the process was not inhibited by mercurials. These differences suggested the participation of an enzyme in the human erythrocyte which may be lacking in the rabbit.

1017. PYRUVATE UTILIZATION IN HEART SARCOSOMES. Oscar K. Reiss (intr. by Leslie Hellerman). Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md.

In connection with studies of cellular pyruvate utilization in this laboratory, and in view of current interest in the metabolic disposition of pyruvate in the myocardium, we have investigated isolated sarcosomes from the point of view of stability in respect to the conversion of pyruvate to citrate, as well as of certain chemical interferences with this process. Sarcosomes were isolated from rat heart by a standard procedure with the use of 0.25 M sucrose - 0.001 M ethylenediaminetetraacetate. Either in phosphate-bicarbonate buffer, pH 7.5, or in tris(hydroxymethyl) aminomethane buffer pH 8.0, it was possible to recover 6 - 7 μM of citrate corresponding to a decrease of 10 μM of pyruvate/mg nitrogen/hr. For such a conversion there is required the addition with respect to pyruvate of an equimolar amount of malate or fumarate plus Mg^{++}, phosphate (if not present in the buffer), and, in addition for optimal results, cytochrome-c and adenosine-5'-phosphate or diphosphate. The sarcosome preparations are sensitive to freezing, but maintain their full activity at 0° for 3 - 4 days. o-Phenanthroline, 10^{-4} M and Ca^{++}, 10^{-3} M are fully inhibitory. Ethylenediaminetetraacetate prevents or reverses the calcium effect. Arsenoso compounds are inhibitory. Fluoroacetate slows pyruvate decrease without affecting citrate accumulation. The nitro compounds, nitrofurazone or chloramphenicol apparently do not interfere. (Supported in part by Natl. Cancer Inst. Grant No. C-392 to Johns Hopkins University.)

1018. ENZYMATIC METHYLATION OF 2,6-DIAMINOPURINE TO 2-METHYLAMINO-6-AMINO-PURINE. Charles N. Remy (intr. by W. W. Westerfeld). Med. Research Div., VA Hosp. and Dept. of Biochemistry, State Univ. of New York, Syracuse.

Resting cell suspensions of Escherichia coli B incubated with 2,6-diaminopurine (DAP), glucose and phosphate were found to contain 2-methylamino-6-aminopurine (MeDAP) in the TCA soluble nucleotide fraction of the cells and in the incubation media. The identification of the isolated base as MeDAP was based on: 1) its formation from DAP-8-C^{14}, 2) a similarity in absorption spectrum with DAP, 3) a consistently higher R than DAP in various solvent systems, 4) incorporation of carbon C^{14} from uniformly labeled glucose into the isolated MeDAP, 5) identification of 2-methylamino-6-hydroxypurine as the deamination product of MeDAP, 6) comparison of the isolated MeDAP with an authentic sample. 5'-phosphoribosyl derivatives of both DAP and MeDAP have been isolated and identified from the TCA soluble nucleotide fraction; the presence of the di- and tri-phosphonucleosides was also indicated. Adenine was not only able to prevent the growth inhibition of E. coli by DAP, but also prevented the incorporation of DAP and MeDAP into the nucleotide fraction of resting cell suspensions. A mutant of E. coli which was resistant to the growth inhibitory effects of DAP was able to convert DAP to MeDAP, but no evidence could be obtained that DAP or MeDAP were incorporated into the nucleotide fraction. The requirements for the conversion of DAP to MeDAP by a crude, cell free enzyme system from E. coli included L-methionine, ATP and magnesium ions. The methyl group of L-methionine-CH_3-C^{14} was incorporated into MeDAP. The DAP and MeDAP nucleotides have also been isolated from this cell free system.

1019. ENZYMATIC SYNTHESIS OF METHYLMALONIC SEMIALDEHYDE. George Rendina (intr. by Henry C. Eckstein). Dept. of Biological Chemistry, Med. School, Univ. of Michigan, Ann Arbor.

β-Hydroxyisobutyrate, an intermediate in valine metabolism, is known to furnish methylmalonic semialdehyde by the action of a specific DPN-dependent dehydrogenase present in extracts of animal tissues, Tetrahymena pyriformis, Neurospora crassa, and Aerobacter aerogenes. It has recently been found that the aldehyde acid can also be generated enzymatically

by transamination between the corresponding amino acid (β-aminoisobutyrate) and α-ketoglutarate. In the course of attempts to establish the metabolic fate of the aldehyde acid evidence has been obtained suggesting a third pathway for the biosynthesis of this compound, by reduction of a methylmalonate derivative. Spectrophotometric determinations indicate that reduced TPN undergoes oxidation in the presence of dialyzed ammonium sulfate fractions of pig kidney on the addition of synthetically prepared methylmalonyl phosphate or methylmalonyl coenzyme A. Free methylmalonate, on the other hand, exhibits no activity in the system, and reduced DPN is less effective than reduced TPN. Methylmalonic semialdehyde, the presumed reduction product, has been prepared synthetically and found to decarboxylate spontaneously to furnish propionaldehyde. It is postulated that methylmalonic semialdehyde formed in the course of valine metabolism may in part be oxidized to methylmalonyl phosphate (or the corresponding coenzyme A thiol ester) by reversal of the reduction step described.

1020. RAT LIVER XANTHINE OXIDASE ACTIVITY IN MOLYBDENUM DEFICIENCY. Dan A. Richert and W. W. Westerfeld. Dept. of Biochemistry, State Univ. of New York, College of Medicine, Syracuse.

The addition of sodium tungstate (94 mg/kg) to a purified casein diet virtually eliminated the xanthine oxidase from rat and chicken tissues when the enzyme activity was measured manometrically. In chicks, half of the purines excreted consisted of xanthine and hypoxanthine, but uric acid and allantoin excretions in rats were unaffected. This could mean that: 1) small amounts of xanthine oxidase escaped detection by the manometric procedure, or 2) uric acid was formed by another pathway in rats. The first possibility was established when small amounts of xanthine oxidase were demonstrated in crude homogenates which registered zero activity manometrically. More hypoxanthine and xanthine were formed in such liver homogenates (presumably from nucleic acids) during the manometric determination than could be oxidized by the small amount of xanthine oxidase present. Since this occurred in the blank vessel as well as in the one containing added substrate, no difference in oxygen consumption between the 2 flasks (hence no xanthine oxidase activity) could be observed. About 0.2 mg of allantoin (determined colorimetrically) was produced by 0.3 gm of such liver (dialyzed homogenate without added substrate) in a 2-hr incubation. This was sufficient activity to account for the normal excretion of 12 - 15 mg of allantoin/100 gm rat/24 hr. The xanthine oxidase inhibitor, 6-pteridyl aldehyde, inhibited this allantoin production by the homogenate. From a study of net oxygen consumption as well as uric acid and allantoin production from various nucleoside and nucleotide substrates by liver homogenates from tungstate-fed rats, there were no indications that xanthine oxidase was by-passed through a conversion of these substrates to uric acid riboside.

1021. DIMINISHED TISSUE UPTAKE OF α-AMINOISOBUTYRIC ACID IN VITAMIN B_6-DEFICIENT RATS. Thomas R. Riggs, Lois M. Walker* and Halvor N. Christensen. Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.

Experiments with isolated cells suggest that pyridoxal participates in active amino acid transport. The present study seeks to evaluate this participation in the intact rat, a task which is complicated because pyridoxal also participates in amino acid degradations and syntheses. Therefore an amino acid has been used which is actively transferred but neither degraded nor anabolized. The distribution of the labeled amino acid could thus be observed by radioactive counting. Rats on normal and vitamin B_6-deficient diets were injected i.p. with 1 mg of carboxyl-labeled α-aminoisobutyric acid/kg body weight and killed 39 hr later following a 15-hr fast. Serum and urine were counted directly; hot water extracts (pH 5-6) were prepared for skeletal muscle, liver, kidney, heart and duodenum. Under these conditions serum activity was elevated from a normal of 1200 cpm/ml to 1700 in B_6-deficiency. Corresponding levels in skeletal muscle, kidney, heart and duodenum were normal or below. Since normally tissue amino acid levels increase in response to increased extracellular levels, the lowered distribution ratios in these tissues in B_6-deficiency is taken to show handicapped transport. Liver radioactivity increased in the deficiency far above that expected from the increased serum levels; possible adrenal cortical effects are being considered. Total urinary loss of the amino acid by deficient rats was 1/4 of normal. All changes were highly significant ($P = 0.02$ or less), and could be reversed by 1 mg of pyridoxine given i.p. along with the α-aminoisobutyric acid.

1022. ISOLATION AND CHEMICAL PROPERTIES OF FLAGELLAR PROTEINS. J. N. Rinker, Toshio Kobayashi and Henry Koffler (intr. by S. M. Hauge). Dept. of Biological Sciences, Purdue Univ., West Lafayette, Ind.

Bacterial flagella are organized aggregates of proteins (recently named "flagellins") that give X-ray diffraction patterns characteristic of the keratin-myosin-epidermin-fibrinogen group. After removal from the cell bodies by shaking, flagella can be isolated in highly purified condition by fractional centrifugation, or a combination of centrifugation and fractional precipitation with ammonium sulfate. Purification by centrifugation is aided by the removal of the "center-bottom" portion of precipitates, which often contains impurities. Experiments involving the stepwise saturation of the suspensions with ammonium sulfate indicated that the 0-0.1 and 0.5-1.0 fractions are the least homogeneous. By discarding them one can obtain fairly purified preparations. To isolate material of the highest purity additional fractionation within the 0.1-0.5 saturation range is needed. Flagella "disintegrate" at pH 2 apparently into individual flagellin molecules or small multiples thereof. These units are no longer centrifugable at the RCF at which whole flagella can be sedimented. In impure preparations a residue remains after centrifugation ("pH 2 insoluble"); this residue contains the bulk of the nonprotein materials of the preparation. Highly purified flagella contain no detectable "pH 2 insoluble" materials, hexose, pentose, nucleic acid, or ash. Flagellin from Proteus vulgaris has the following empirical formula (moles/10 gm of protein): Lys_{46} Arg_{37} Asparagine$_{136}$ Asp_{21} Glu_{92} $Custine_3$ $Meth_7$ Tyr_{12} Ser_{64} Thr_{81} Gly_{81} Ala_{104} Val_{81} Leu_{81} $Isoleu_{53}$ $Phenylala_{23}$ (the amide-N is arbitrarily represented as asparagine). Because of the possibility that the flagellins are contractile proteins, the response of flagella (as macrofibers, prepared by compression of surface films, and in suspension) to the addition of adenosinetriphosphate was studied. Neither contraction nor "superprecipitation" could be demonstrated under a variety of conditions, including those under which actomyosin exhibited these phenomena.

1023. EFFECT OF SOME GLUTAMINE ANTAGONISTS ON ALCOHOL CONSUMPTION BY RATS. Lorene L. Rogers and Richard B. Pelton (intr. by Roger J. Williams). Biochemical Inst. and the Clayton Fndn. for Research, Univ. of Texas, Austin.

Earlier studies have shown that the addition of glutamine to the diet of laboratory rats reduces their voluntary alcohol intake and that this effect is not duplicated by a number of related amino acids (Rogers et al. J. Biol. Chem. 214:503, 1955; 220:321, 1956). Some reported glutamine antagonists have now been added to the diets of rats and their effects on alcohol consumption studied. Azaserine at a level of 750 μg/rat/day produced only a slight increase in alcohol consumption. On the other hand when 50 mg/rat/day of methionine sulfoximine was fed for only 2 days, a relatively sharp increase in consumption was observed during the 1st wk followed by a persistent drop below the control levels. The administration of 300 mg/rat/day of glutamine along with the methionine sulfoximine did not appreciably alter this drinking pattern. Methionine sulfoximine has also been administered at levels of 100 mg for a 2-day period and 25 mg for 26 days, and methionine sulfoxide has been administered at the 50 mg level. The possible relationship of these results to appetite for alcohol will be discussed.

1024. STIMULATION OF THE CYANIDE-SENSITIVE ALKALINE PHOSPHATASE OF RAT LIVER BY PROTEIN DEFICIENCY AND DIETARY FAT. Otto Rosenthal, Elena Armocida,* Vincent Coyle,* Harry M. Vars and John Hobart.* Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.

We have previously reported that the amount of particle-bound, cyanide-sensitive alkaline phosphatase of liver greatly increases after partial hepatectomy and during protein fasting. Since both the cyanide-sensitive plasma phosphatase, which originates in the small intestine, and bile phosphatase rise after fat feeding the effect of dietary fat on the liver phosphatase was explored. Four semisynthetic diets containing 18% casein, 20% zein, 18% gelatin or no protein were fed for 2 wk to groups of 6 rats without added fat and with 3 and 12% cod-liver oil respectively. In the casein, zein and gelatin groups the phosphatase content of the livers increased with the fat content of the diet. The greatest increase occurred in the zein group. In contrast, the liver phosphatase content of the no-protein group while consistently elevated was not augmented by fat absorption. As to the increase of the plasma phosphatase levels by dietary fat no marked differences were observed among the groups. There exists thus a correlation between the activity of the insoluble, cyanide-sensitive hepatic phosphatase and fat absorption such as holds for the soluble phosphatases of plasma and bile. Response of the hepatic phosphatase, however, is modified by type and amount of protein supplied. These interrelationships will be discussed and supplementary data on other liver phosphatases presented. (Supported in part by Army Contract DA-49-007-MD-143 and an Institutional Grant from the American Cancer Society.)

1025. PARTIAL PURIFICATION AND CHARACTERIZATION OF RIBONUCLEASE INHIBITOR FROM RAT LIVER. Jay S. Roth. Div. of Biological Chemistry, Hahnemann Med. College, Philadelphia, Pa.

A new assay for RNase inhibitor has been devised that allows determination of bound RNase and also inhibitor capacity for inactivating added crystalline pancreatic RNase. Bound RNase is liberated by 4 x 10^{-4} M p-chloromercuribenzoate (CMB). Pancreatic RNase is added to another aliquot and inhibition determined. A plot on semilog coordinates of percentage inhibition versus concentration of added pancreatic RNase gives a straight line of constant slope; the 50% inhibition intercept on the abscissa is directly proportional to the amount of inhibitor. Purification of 4-6 times with respect to bound RNase and 3-5 times with respect to inhibiting activity has been achieved, thus far, by salt precipitation followed by lyophillization. The effect of sulfhydryl inhibitors on the purified preparations has been determined. Maximum release of RNase activity is obtained between 8 x 10^{-5} and 2 x 10^{-3} M concentrations of CMB. At lower concentrations release of nuclease activity rapidly falls off and at higher concentrations there is some decrease in enzyme activity possibly due to inactivation. p-Mercuribenzenesulfonic acid gives essentially the same results but iodoacetamide is inactive below 5 x 10^{-3} M concentration and phenyl arsine oxide is about 1/2 as active as CMB. Cysteine does not reverse the action of CMB but absorption of the sulfhydryl reagent on added ion exchange resin will do so under certain conditions. Inhibitor activity can be prevented by the presence of an insoluble polysaccharide obtained from yeast. The action of the polysaccharide, as well as salts and certain metal ions on RNase inhibitor will be discussed. (Aided by grants from the American Cancer Society, Natl. Insts. of Health and Damon Runyon Memorial Fund.)

1026. INTERMEDIATES BETWEEN 2-KETO-3-DEOXY-7-PHOSPHOGLUCO-HEPTONIC ACID (KDPH) AND 5-DEHYDROQUINIC ACID (DHQ). Joel Rothschild (intr. by G. L. Foster). Dept. of Biochemistry, Columbia Univ., New York City.

Conversion of D-erythrose-4-phosphate plus phosphoenolpyruvate to intermediates in aromatic biosynthesis has been postulated to proceed by way of an initial condensation yielding KDPH. This compound has been obtained chromatographically pure by unambiguous chemical synthesis starting with 2-deoxy-D-glucose, and was found to be quantitatively converted to DHQ by cell-free extracts of Escherichia coli. In crude extracts 10^{-2} molar Versene inhibits the synthesis of DHQ from KDPH. One mole of inorganic phosphate is released and a new intermediate is formed which is still an α-keto acid. Work on the structure of this intermediate is in progress and will be reported.

1027. SOME PROPERTIES OF A LIVER T_3 AND T_4 DEIODINASE SYSTEM. W. R. Ruegamer and Stuart Yunis.* VA Hosp. and State Univ. of New York College of Medicine, Syracuse.

A survey of the major tissues of the rat revealed that the liver possesses the greatest deiodinase activity for triiodothyronine (T_3) and Tetraiodothyronine (T_4). From temperature, pH and time dependence studies, it was observed that maximal deiodination takes place with constant shaking in 3 hr at 37°C and a pH of 5.8. Using 15 gammas of substrate/10 ml of buffer, it was found that 40 mg of fresh liver homogenate would deiodinate approximately 25% of the substrate present, and that further additions of liver had no significant effect upon the amount of substrate deiodinated. However it was observed that the reaction rate follows the established kinetic theory for enzyme reaction between substrate concentrations of 0.1 and 20 gammas and enzyme concentrations of 15 - 50 mg of liver/10 ml of medium. Therefore, 100 U of enzyme activity were arbitrarily defined as that amount of enzyme which would deiodinate 25% of the substrate (25 gammas) in 3 hr under the conditions described. The enzyme activity is present in the particulate matter as determined by centrifugation studies, and appears to be readily destroyed by oxidation. A wide variety of chemical substances will inhibit the reaction and others will protect, although there appears to be no

definite pattern established in this respect. Fractionation procedures are being employed in an effort to obtain the system in a purified state so that the deiodination reaction can be studied at the molecular level.

1028. IN VIVO RELEASE OF AMINO ACIDS FROM AN INTACT PROTEIN. Herbert W. Rumsfeld, Jr., William W. Burr, Jr. and Donald S. Wiggans (intr. by Herbert C. Tidwell). Biochemistry Dept., Univ. of Texas Southwestern Med. School, Dallas.

The ability of a protein to exchange amino acids with those of the surrounding medium, perhaps on a template at the site of protein synthesis, has been the subject of a number of investigations. In a previous report (Fed. Proc. 15:384, 1956), utilizing valine-1-C^{14} and methionine-S^{35}, this concept was supported. In the present study, valine-1-C^{13} and leucine-1-C^{14} have been given intraperitoneally to a donor rat. At the end of an 8-hr biosynthetic period, the rat was exsanguinated by cardiac puncture, and electrophoretically pure serum albumin was isolated. This protein was given i.v. to a recipient animal, and after 2 days pure albumin was again isolated. The C^{13}/C^{14} ratios of the albumin before and after passage through the recipient rat were compared. The results show a marked difference in the rates of release of the 2 isotopes. Approximately a 30-fold increase in the ratio was observed suggesting that the C^{14} label was leaving the protein at a more rapid rate. This would be compatible with the theory that proteins may exchange amino acids without an obligatory complete breakdown of the molecule. (Supported by grants from the Robert A. Welch Fndn., Houston, Tex., and the Atomic Energy Comm. Contract No. AT-(40-1)-1988.)

1029. BIOCHEMICAL STUDIES OF ANIMAL CELLS GROWN IN TISSUE CULTURE. W. J. Rutter, R. W. Brosemer and O. C. Richards (intr. by Wm. C. Rose). Div. of Biochemistry, Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.

Certain aspects of the metabolism of various animal cells maintained in tissue culture have been investigated. Comparisons between cell strains, and between parental and "altered" lines have been made with respect to the following parameters: respiration, aerobic and anaerobic glycolysis, and the metabolism of substrates. The effects of metabolic regulators (e.g., insulin, thyroxine, and growth hormone) on the metabolism of the cells will also be discussed.

1030. CEREBRAL METABOLISM OF BUTYRATE-1-C^{14} IN NORMAL HUMAN SUBJECTS. William Sacks (intr. by Cecilia Riegel). Research Dept. of Albert Einstein Med. Ctr., Philadelphia, Pa.

Previous studies of cerebral glucose metabolism in vivo (J. Appl. Physiol. In press) suggested that there is a constant production, by the brain, of CO_2 from some source(s) other than glucose. In attempting to find other sources of cerebral CO_2 it was decided to employ certain lipid and protein intermediates as substrates. One of the lipid intermediates, butyrate-1-C^{14}, will be the subject of this report. The total activity of labeled CO_2 in the blood entering and leaving the brain was determined following a single injection of butyrate-1-C^{14} in normal human subjects. The collection of samples as well as the determination of total CO_2 activity in each specimen were done as previously described (J. Appl. Physiol. 9:43, 1956). An estimation of the fraction of the cumulative $C^{14}O_2$ resulting from the oxidation of labeled butyrate, over a 90-min time interval following injection, showed that an average of 3.9% of the injected C^{14} was converted into $C^{14}O_2$, by the brain. A comparison of the "specific activities" of the "added increment" $C^{14}O_2$ (i.e. the "specific activity" of the CO_2 produced by the brain and added to the venous blood leaving the brain) with butyrate "specific activities" of venous blood showed that from 2 to 8% of the "added" CO_2 was derived from butyrate. Investigations using another lipid intermediate, glycerol-C^{14}, as substrate are in progress and will be discussed. (Supported by Grant M-789, from the Natl. Inst. of Mental Health, Natl. Insts. of Health, PHS and by Grant S-15 from the Albert Einstein Med. Ctr.)

1031. L-KYNURENINE HYDROXYLASE. Yoshitaka Saito,* Osamu Hayaishi, Simon Rothberg* and Shiro Senoh.* Natl. Insts. of Health, Bethesda, Md.

De Castro, Price and Brown (J. Am. Chem. Soc. 78:2904, 1956) reported that 3-hydroxykynurenine and kynurenic and xanthurenic acids were produced from L-kynurenine by rat or cat liver mitochondria in the presence of TPN, citrate and nicotinamide. When citrate was replaced by glucose and glucose dehydrogenase, the yield of 3-hydroxykynurenine was consistently higher and the formation of kynurenic and xanthurenic acids became almost negligible. A partially purified enzyme preparation of L-kynurenine hydroxylase has now been obtained from rat liver mitochondria by treatment with 10 KC Raytheon sonic oscillator followed by centrifugation at 100,000 g for 1 hr and by fractionation with alkaline ammonium sulfate. The enzymatic activity can be determined spectrophotometrically by measuring the rate of oxidation of TPNH at 340 mμ. The activity was enhanced 5-10 fold by the addition of cyanide or azide but was not affected by antimycin or ethylenediaminetetraacetate. The enzyme has maximum activity at pH 8.0 and was specific for L-isomer of kynurenine. The rate of oxidation of TPNH with o-hydroxy- and o-nitrobenzoylalanine was approximately 50% of that with L-kynurenine. None of the following compounds was oxidized at an appreciable rate: 5-hydroxykynurenine, formylkynurenine, kynuramine, o-aminobenzoylpropionic acid, benzoylalanine, N -acetylkynurenine, kynurenic acid and anthranilic acid. Experiments with H_2O^{18} and O_2^{18} indicate that the oxygen atom incorporated into 3-hydroxykynurenine, was derived from atmospheric oxygen and not from the oxygen atom in the water molecule. Kynurenine hydroxylase, therefore, represents another example of an oxygenase which utilizes and incorporates atmospheric oxygen into a substrate.

1032. APPARENT FREE AMINO ACIDS IN DEPROTEINIZED PLASMA OF NORMAL AND OF UREMIC DOGS AND HUMANS. Peter F. Salisbury,* E. A. Murphy* and Max S. Dunn. Chemical Lab., Univ. of California, and Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles.

Heparinized plasma was procured by exsanguination from normal, fasting dogs and from nephrectomized dogs with severe uremia. Plasma was also procured from nine healthy, fasting young men and from 6 patients with severe uremia due to chronic glomerular nephritis. The concentration of 13 amino acids in the protein free extracts from these plasmas was determined with standard microbiological methods. The concentration of arginine and of glycine was significantly increased in the plasma of uremic dogs. Concentrations of methionine and of threonine were decreased in uremic dog plasma. Glycine, proline and arginine were increased and methionine, threonine,

leucine, isoleucine and lysine were decreased in uremic human plasma. There exists the possibility that certain clinical features of the uremic syndrome may be related to abnormal plasma amino acid levels. This possibility will be discussed.

1033. MECHANISM OF COPPER ACCUMULATION BY LIVER SLICES. Paul Saltman, T. Alex and B. McCornack (intr. by Arnold Ware). Dept. of Biochemistry, Univ. of Southern California, Los Angeles.

Studies in our laboratory concerning the mechanism for iron accumulation by rat liver slices and cell suspensions indicated that iron is taken up against an apparent concentration gradient by a process not directly coupled to metabolic energy. Kinetic experiments demonstrated that some specific sorption mechanism is mediating this accumulation. We have now turned our attention to copper metabolism and have found a similar mechanism is involved. Liver slices were incubated in Krebs-Ringer phosphate buffer containing 2 γ/ml of copper as cupric citrate. The total copper concentrations in the slice were determined by the dithizone method after suitable digestion of the tissue. The copper concentration initially was 3 γ/gm wet weight and reached a maximum level of 16 γ/gm after 90 min. This accumulation as not affected by azide, dinitrophenol, CN^-, anaerobiosis, or other metabolic inhibitors. That copper is sorbed at specific sites was demonstrated by both kinetic studies as well as by adsorption isotherm experiments using different external copper concentrations. Unlike the iron system, the copper system did not seem to involve essential -SH groups at the site. The energy of activation for copper accumulation is of the order found for a rate process where a diffusion step is limiting. It would appear by all of the criteria examined that the mechanisms for copper and iron accumulation are fundamentally the same and constitute a means whereby ions can be accumulated without the direct expenditure of metabolic energy. (Supported in part by the Atomic Energy Comm. and by Smith, Kline & French Fndn.)

1034. PRECURSORS OF THE BASES OF RNA AND DNA STUDIED IN TISSUE CULTURE. N. P. Salzman and E. D. Sebring (intr. by Ben Bloom). Natl. Insts. of Health, Bethesda, Md.

A human epithelial carcinoma cell (HeLa) maintained in tissue culture did not require preformed purines or pyrimidines for growth, but did require glutamine. The glutamine could be replaced by glutamic acid only when the latter was added at 20 times the optimal growth concentration of glutamine. The role of glutamine as a precursor of the bases of RNA and DNA was studied, using glutamine-C^{14}, glutamine-amide-N^{15}, glutamic acid-C^{14}, and $N^{15}H_4Cl$ as precursors. Both N^{15}-containing precursors were shown to be effectively incorporated into the purines and pyrimidines of RNA and DNA. Glutamine-C^{14} and glutamic-C^{14} were effective precursors of the pyrimidines, but labeled the purine bases only slightly. The origin of the amino groups in adenine and guanine in this system was also studied. There appear to be differences between this system and others thus far reported with regard to the source of the nitrogens of the purine ring.

1035. PASSAGE OF CALCIUM AND STRONTIUM IONS THROUGH MEMBRANES. Joseph Samachson, Herta Spencer, Bernard Kabakow and Daniel Laszlo (intr. by Irwin B. Wilson). Div. of Neoplastic Diseases, Montefiore Hosp., New York City.

Discrimination in favor of calcium against strontium has been found with regard to absorption in plants, animals and man. This discrimination plays an important role in assessing the toxicity of radiostrontium to man. It was of interest to study whether it is related to the physical chemical properties of the ions involved such as the ability to pass through membranes. Ca^{45} and Sr^{85} were added to serum and the passage of calcium and strontium ions through Visking casings was studied in vitro. A higher percentage of strontium than of calcium filtered through the membrane. Almost invariably, also, filtration of both ions was greater at room temperature (about 22-25°C) than at body temperature. In vivo studies were also made of the transfer of calcium and strontium from plasma to other body fluids, such as peritoneal and cerebrospinal fluid. Different transfer rates were noted for calcium and strontium from plasma to peritoneal fluid, these rates being apparently dependent on the protein content and composition of the various fluids. The transfer of these ions from plasma to cerebrospinal fluid was studied in 10 patients. The calcium content of the fluid was approximately 55% of the calcium present in the plasma, whereas the ratio of strontium was significantly lower. The implication of these results with regard to protein binding of calcium and strontium, excretion of strontium, and deposition of radioactive strontium in bone will be discussed.

1036. REACTIONS OF THIOCTIC ACID AND THIOCTAMIDE CATALYZED BY THE α-KETOGLUTARIC DEHYDROGENASE COMPLEX. D. R. Sanadi and Robert I. Searls.* Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

Mammalian α-ketoglutaric dehydrogenase is an enzyme complex of molecular weight 2 million and contains tightly-bound diphosphothiamine, thioctic acid (or a derivative) and magnesium. It appears, under the electron microscope, as a nearly spherical particle of 120 Å diameter. Besides the oxidation of α-ketoglutarate (KG) by DPN, the enzyme complex catalyzes the reduction of 6,8-thioctic acid and of 6,8-thioctamide by reduced DPN (DPNH). The reaction is reversible and can be followed readily in either direction by measuring the absorption of DPNH at 340 mμ (+) thioctic acid is active in the reaction while (-) thioctic acid is inactive. The pH optima for KG oxidation and thioctamide reduction are between 7.0 and 7.4. With thioctic acid, however, the activity increases with decreasing pH from 7.0 to 6.0. The apparent K_m for DL-thioctamide is 6×10^{-4} M while maximal rates are not obtained with DL-thioctate even at 4×10^{-3} M. The ratios of the rates of KG oxidation : thioctate reduction (at suboptimal conditions) : thioctamide reduction are as 1 : 1.3 : 4.8. The data on pH optima and K_m would tentatively suggest that thioctamide is closer in structure to the natural bound cofactor than thioctic acid. The redox potential of the thioctic acid couple calculated from the equilibrium constant of the reaction of dithioloctanoic acid with DPN is -0.307.

1037. BIOSYNTHESIS OF ESTRONE AND PROGESTERONE IN THE PREGNANT MARE. Kenneth Savard, Karl Andrec,* B. W. L. Brooksbank,* Cesare Reyneri,* Ralph I. Dorfman, R. D. H. Heard,* Ross Jacobs* and Samuel S. Solomon.* Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass., and Dept. of Biochemistry, McGill Univ., Montreal, Canada.

Isolation by conventional means and measurement

of radioactivity of estrone and 3β-hydroxyallopregnan-20-one from the daily urines (days 1 - 10) following the administration (i.v.) of acetate-1-C^{14} to a pregnant (9th month) mare, revealed both compounds to have similar rates and extent of isotope incorporation over the experimental period. Calculations based on the molar activity (counts per millimole) of both compounds suggest that estrone and progesterone are derived, via a common precursor, from acetate at similar rates. The calculated number of labeled-carbon positions in estrone (but not in equilin nor equilenin) approximates numerically that which is predictable from the isotope distribution in cholesterol biosynthesized from acetate-1-C^{14} (Bloch and collaborators; Cornforth and Popjak). Other radioactive neutral steroids found were allopregnane-3β, 20 α-diol, allopregnane-3β, 20β-diol and allopregnane-3, 20-dione, which suggest that in the mare the catabolism of progesterone (saturation of ring A) is directed towards the 5 trans (α) orientation, since pregnane-3, 20-dione and pregnane-3 α, 20 α-diol were not found (by carrier dilution). The methods used were the conventional fractionation procedures and included separation with Girard's reagent, digitonin and column chromatography; radiochemical purity of the neutral steroid samples was achieved by recrystallizations, formation of derivatives and paper partition chromatography.

1038. INHIBITION OF ELECTRON TRANSPORT IN ESCHERICHIA COLI BY AUREOMYCIN. A. K. Saz, L. M. Martinez and M. B. Middleton (intr. by H. G. Steinman). Natl. Insts. of Health, Bethesda, Md.

Previous reports from this laboratory have shown that the reduction of aromatic nitro compounds to nitroso compounds mediated by extracts of aureomycin-sensitive Escherichia coli is inhibited by low concentrations of the antibiotic. The nitro reductase of aureomycin-resistant cells derived from the sensitive cells was resistant to the antibiotic. Both enzymes were presumably metallo-flavoproteins, and in both instances, aureomycin inhibited the transfer of electrons from reduced conjugated flavin to the nitro group. The present communication reports the effect of aureomycin on other electron transport systems. Extracts which reduced both 2,6-dichlorophenol indophenol and ferricyanide were prepared from aureomycin-sensitive and -resistant cells. Phenol-indophenol reduction catalyzed by the extract derived from the sensitive cell was inhibited by low concentrations of aureomycin. Considerably higher concentrations of the antibiotic were needed to produce similar inhibition of the enzyme derived from the resistant cell. Ferricyanide reduction was inhibited equally in both sensitive and resistant extracts. This paradoxical result is apparently related to the fact that ferricyanide reduction, in distinction to both nitro and phenol-indophenol reductions, is a one electron reduction. Further purification of the sensitive enzyme yielded two fractions both mediating similar reductions. One fraction was sensitive to the antibiotic, the other was resistant. This observation may explain the fact that aureomycin is bacteriostatic rather than bactericidal, since a bypass of an inhibited reaction is presumably present in the intact cell.

1039. 5-FLUOROPYRIMIDINES AS GROWTH INHIBITORS OF MICROORGANISMS. J. M. Scheiner, E. Kostelak and R. Duschinsky (intr. by Lowell O. Randall). Hoffmann-La Roche Inc., Nutley, N. J.

Fluorinated pyrimidines were investigated as antagonists of natural pyrimidines and were found to display tumor-inhibitory activity through interference with nucleic acid metabolism (Heidelberger et al. Nature. To be published). The present report offers antimicrobial data obtained with fluoropyrimidines. The compounds were synthesized by Duschinsky, Pleven and Heidelberger. 5-Fluorouracil (Ro 2-9757), when tested in semisynthetic media, has been found to be a potent inhibitor of growth of Lactobacillus leuchmannii (0.0048), Lactobacillus arabinosus (0.018), Streptococcus faecalis (0.00024), Lactobacillus casei (0.00035), Saccharomvces carlsbergensis (0.057), the concentration in meg per ml of 5-fluorouracil required for one-half maximum inhibition of growth being indicated in parenthesis. Comparative figures obtained with L. leichmannii are for 5-fluorocytosine 0.038, 5-fluoroorotic acid 0.20, 2-thio-5-fluorouracil 4.5, 5-bromouracil 11.0 and 6-azathymine > 10. Thymidine, thymine, 5-methylcytosine in order of decreasing activity are more effective than the nonmethylated pyrimidines, uracil and cytosine, in reversing the inhibition caused by 5-fluorouracil. The almost total inhibition of L. leichmannii by 0.01 mcg/ml fluorouracil is reversed to one-half maximum growth by 0.2 mcg/ml thymidine. A 10-fold increase of fluorouracil requires the same amount of thymidine for reversal, which indicates that the relationship is noncompetitive. Folic acid and vitamin B_{12} in relatively large amounts have little effect on the fluorouracil inhibition. Strains of L. leichmannii and L. arabinosus resistant to the inhibitory action of fluorouracil have been produced.

1040. EFFECT OF HYPOPHYSECTOMY AND GROWTH HORMONE ON TURNOVER OF ACID MUCOPOLYSACCHARIDES IN RAT SKIN. Sara Schiller and Albert Dorfman. LaRabida Jackson Park Sanitarium, and Dept. of Pediatrics, Univ. of Chicago, Chicago, Ill.

The influence of hormones on the metabolism of the mucopolysaccharides, hyaluronic acid (HA) and chondroitinsulfuric acid (CSA), is being studied in order to evaluate their role in the regulation of mucopolysaccharide turnover. The turnover of HA and CSA in skin was estimated in normal, hypophysectomized and growth hormone-treated hupophysectomized rats utilizing acetate-1-C^{14} and $Na_2S^{25}O_4$. Half-life times were calculated from the specific activities 1, 3, 5 and 9 days after the administration of the radioactive mixture. In normal animals, half-life times of 2.9 and 4.8 days, respectively, were found for HA and CSA. After hypophysectomy, the rate of C^{14} decay in the mucopolysaccharide fractions was decreased, as indicated by half-life times of 5.1 days for HA and 11.7 days for CSA. When hypophysectomized rats were injected with growth hormone, the half-life time of HA was 7.9 days while that of CSA was 5.5 days. The results demonstrate decreased turnover of both HA and CSA fractions in the absence of the pituitary gland. However, only the CSA turnover is restored by the administration of growth hormone; HA turnover remains unaffected. The differential effect of growth hormone on metabolism of mucopolysaccharides may indicate specific functions for the individual substances and may explain the selective effects of hormones on target organs. (This work was supported by the Herman Gordon Fndn.)

1041. NATURE OF THE REACTION OF DOG INTESTINAL ALKALINE PHOSPHATASE WITH ITS ANTIBODIES. Max Schlamowitz. Div. of Biochemistry, Roswell Park Mem. Inst., Buffalo, N. Y.

The formation of antibodies against the alkaline

phosphatase from dog intestine has been previously demonstrated (Schlamowitz. J. Biol. Chem. 206:361, 1954). The belief that the antigenic and catalytic sites on the enzyme are not identical now receives further support from the facts that treatment of the enzyme with acetic anhydride destroys its ability to precipitate with antibody but leaves the enzymatic activity largely intact, while treatment of the enzyme with cysteine destroys the catalytic activity of the enzyme but leaves its antibody-combining ability intact. In the region of excess antibody dog intestinal phosphatase is not completely precipitated by its antiserum. The addition of either horse antirabbit γ-globulin antibody or of cysteine-inactivated enzyme to such systems causes complete precipitation of the phosphatase. These facts are taken as evidence for the formation of soluble complexes of antigen and antibody in the region of excess antibody in the dog intestinal phosphatase-rabbit antiserum system. Treatment of the antibody with acetic anhydride markedly affects its reaction with the enzyme.

1042. PREPARATION AND ISOLATION OF FATTY ACIDS RANDOMLY LABELED WITH C^{14}. Hermann Schlenk and Helmut K. Mangold (intr. by W. F. Geddes). Hormel Inst., Univ. of Minnesota, Austin.

Various problems in the study of fat metabolism make desirable the use of long chain fatty acids randomly labeled with C^{14}. This pertains in particular to unsaturated fatty acids, the chemical synthesis of which is practical only for their carboxyl-labeled form. Radioactive fat was obtained from Chlorella pyrenoidosa grown in a 24-liter flask containing 5 l. of culture medium. The algae were allowed to grow for 6 wk in a closed system and were supplemented with 3 portions of CO_2 the second one containing 7 mc of $C^{14}O_2$, all of which had been absorbed. The cells were harvested and boiled with alkali. A total of 0.2 gm of unsaponifiables and 2.9 gm of mixed fatty acids was obtained, which represents a radioactive yield of approximately 40%. The radioactive acids were diluted with cold fatty acids of Chlorella pyrenoidosa and reacted with diazomethane, yielding a total of 7.8 gm methyl esters. This material was distilled through a spinning band column and 1.3 gm of pure C_{16} and 4.2 gm of pure C_{18} esters were obtained. Fractions containing more than one chain length were combined and hydrogenated. After saponification of the esters, the individual acids were isolated by countercurrent distribution in the system heptane/methanol-formamide-acetic acid (Ahrens and Craig. J. Biol. Chem. 195:299, 1952). The lipids of these algae contain C_{16} and C_{18} acids and no other chain length is found to any extent. Palmitic and stearic acids of the hydrogenated sample were separated by 600 transfers. The series steatic, oleic, linoleic, linolenic acids of the C_{18} sample were separated by 420 transfers, and palmitic, palmitoleic, palmitolinoleic, palmitolinolenic acids of the C_{16} sample of 300 transfers. Each acid was esterified again and distilled, the total recoveries from distribution and distillation being 70-80%. Paper chromatography was of decisive help in all purification steps (Schlenk et al. J. Am. Oil Chemists' Soc. In press). (Supported by Atomic Energy Comm., Contract AT(11-1)-236.)

1043. CHROMATOGRAPHIC SEPARATION OF ACIDIC PROTEINS ON WEEKLY ACID CATION EXCHANGE RESINS. K. Schmid and Margaret B. MacNair.* Robert W. Lovett Mem. Labs., Massachusetts General Hosp., and Dept. of Medicine, Harvard Med. School, Boston.

The chromatographic fractionation of proteins with alkaline or neutral isoelectric points has been reported (Fraenkel-Conrat. Ann. Rev. Biochem. 25:292, 1956). This note describes the separation of proteins with acid isoelectric points (pI) on a week cation exchange resin. Orosomucoid (MW = 45,000; pI 2.7), α_2-glycoprotein (MW = 40 - 50,000; pI 4.0) and serum albumin (pI 4.9) were investigated on Amberlite IRC-50 columns. The "total volume of effluent" (Boardman and Partridge, 1956) of these proteins was determined over a pH range from 4 to 7 and at ionic strengths between 0.06 and 0.75 using acetate, citrate and phosphate buffers. A characteristic pH was found for each of the 3 proteins below which they were retained on the column: albumin at pH 5.8, α_2-glycoprotein at pH 5.0 and α_1-acid glycoprotein (orosomucoid) at pH 4.5. The separation of the 3 proteins from each other was achieved as follows: application of the mixture in pH 5.2 citrate with simultaneous removal of orosomucoid, elution of the α_2-glycoprotein with pH 5.7 citrate and of albumin with pH 6.2 acetate buffer. Further application of this procedure to the purification of orosomucoid (immunochemically homogeneous) and of an α_2-glycoprotein, and to the separation of the proteins of fraction VI from normal human plasma will be discussed. (Grants in support of these investigations received from the Helen Hay Whitney Fndn., N. Y. and from the Natl. Insts. of Health, A-509C2.)

1044. PARTITION OF TISSUE PHOSPHOLIPIDES ON THE BASIS OF THE SOLUBILITY PROPERTIES OF THEIR PHOSPHORUS-CONTAINING SAPONIFICATION PRODUCTS. Gerhard Schmidt and W. A. Spencer.* Boston Dispensary and Biochemistry Dept., Tufts Univ. School of Medicine, Boston, Mass.

The interpretation of the figures for monoaminophosphatides and for sphingomyelin obtained with the procedure of Schmidt et al. (J. Biol. Chem. 166:505, 1946) has been reinvestigated. Improved procedures and new criteria were applied. The alkali-resistant phospholipide fraction was isolated under conditions preserving the bond between lipide aldehydes and the phosphorus-containing moieties of acetal phospholipides. Sphingomyelin was determined in this fraction as well as in the total phospholipide fraction by difference between the amounts of free choline found after hydrolysis with 0.25 N barium hydroxide and with N hydrochloric acid, respectively. (The former procedure yields 95% of sphingomyelin choline and 100% of lecithin choline, the latter no choline from sphingomyelin but all choline from lecithin.) Absence of monophosphatides in the "alkali-resistant fraction" was ascertained by its failure to give a hydroxamic acid. The amounts of sphingomyelin phosphorus was smaller than the difference between the total phosphorus of the "alkali-resistant" fraction and that of the acetal phospholipides. After removal of the acetal phospholipides, the remaining phospholipides are almost completely recovered in the ether-insoluble fraction. The N-, P-, and choline N-distribution of this fraction and its behavior under various conditions of hydrolysis supports the assumption that at least half of it consists of a phosphosphingolipide which does not contain choline.

1045. RNA BIOSYNTHESIS IN RAT LIVER HOMOGENATES. John H. Schneider* and Van R. Potter. McArdle Mem. Lab., Univ. of Wisconsin, Madison.

Whole homogenates of normal rat liver incorporate radioactivity from orotic acid-6-C^{14} into RNA but not DNA. Separation of cell fractions at various times after incubation of whole homogenates indicate that the final supernatant fraction and nuclei contain RNA with the highest specific activity. Microsomal RNA is

much less radioactive. When 4 μ moles of ATP are added at any time during an incubation there is an immediate inhibition of incorporation which is reversed on continued incubation. Studies of the acid-soluble fraction indicate that this reversal is due to a decrease in the level of phosphorylation of the nucleotides. After 45-60 min of incubation, RNA is maximally labeled. On further incubation, the specific activity decreased rapidly. This decrease is coincident with marked degradation of nucleotides in the acid-soluble fraction. Changes in the absolute amount of RNA which may occur during the incubation are too small to be measured. A decrease in the specific activity of RNA also occurs when microsomes with labeled RNA are incubated in a nonradioactive homogenate. Microsomes labeled in vitro or in vivo (30 min after injection) show this effect. Microsomes labeldd for longer periods in vivo do not exhibit this phenomenon. Further evidence for nonuniform labeling was obtained by mild acid hydrolysis which selectively removes the radioactive nucleotides from RNA. There is a progressive activation or stimulation of the incorporating system if a whole homogenate or the cytoplasmic fraction thereof is preincubated at 0°C. A maximum activity is reached by 4 hr and no change occurs for another 20 hr.

1046. A NEW PATHWAY FOR THE METABOLISM OF ETHANOL. Martin P. Schulman and W. W. Westerfeld. Dept. of Biochemistry, State Univ. of New York, College of Medicine, Syracuse.

Alcohol is currently believed to be metabolized by oxidation to acetaldehyde, conversion of the latter to acetyl CoA (either directly or by way of acetate), and complete burning of acetyl CoA through the citric acid cycle. The studies reported below suggest another pathway for the conversion of ethanol to acetyl CoA. Malonate,-inhibited rat liver homogenates were incubated with ethanol-1-C^{14} or acetate-1-C^{14}. Acetoacetate and β-hydroxybutyrate carrier were then added, proteins precipitated, carrier acetaldehyde added to the protein-free filtrate and the latter extracted with ether at pH 7 to remove acetaldehyde. The acetoacetate and β-hydroxybutyrate were individually decomposed into acetone (C-2, 3, 4) and CO_2 (C-1) by modification of Van Slyke's procedure. In agreement with the results of others, acetate-1-C^{14} labeled equally to carboxyl and carbonyl groups of acetoacetate (C-1 and C-3, respectively). C-3 of acetoacetate formed during incubation with ethanol-1-C^{14} contained from 10 to 25 times as much isotope as C-1. Insignificant C^{14} was found in β-hydroxybutyrate derived from acetate-1-C^{14}; β-hydroxybutyrate derived from ethanol contained appreciable C^{14}, and like acetoacetate, most of the isotope was in C-3. The predominant concentration of isotope in C-3 of β-hydroxybutyrate and acetoacetate formed from ethanol-1-C^{14} suggests a condensation of acetaldehyde-1-C^{14} with unlabeled endogenous acetyl CoA to form β-hydroxybutyrate-3-C^{14} (or its CoA derivative). This was probably formed prior to acetoacetate, since C^{14} was found in both products when starting with ethanol-1-C^{14}, but only in acetoacetate when starting with acetate-1-C^{14}. The above studies show that the metabolism of ethanol can proceed by condensation to a 4-carbon compound and subsequent cleavage to acetyl CoA rather than by direct conversion of acetaldehyde.

1047. BENCE-JONES AND MYELOMA PROTEINS. J. Schultz, G. Grannis,* H. Kimmel,* and H. Shay.* Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.

Analysis of certain amino acids of the serum proteins lead to the proposition that the proteins of the serum have an intimate relation reflecting a common origin (Arch. Biochem. 57:174, 1955). Examination of the Bence-Jones and myeloma proteins of a series of patients indicated that these proteins also have a familiar relationship with each other and possibly to the serum proteins (Fed. Proc. 15:350, 1956; Abstr., 130th Meeting, Am. Chem. Soc., 6c, 1956). The discovery of polypeptides with the same amino acid sequence common to these proteins would offer data in support of the above proposition. In this respect, the partial hydrolysates of the myeloma protein and Bence-Jones protein of the same individual have been compared by a number of techniques. In one of these the hydrolysis was carried out in dilute acid to the release of 100% of the aspartic acid. The partial hydrolysate was precipitated with TCA; the soluble portion was called (S-1); after the precipitate was washed with 3:1 alochol-ether, the residue was referred to as R-1; the washings were treated with ether; the ether soluble, E-s, and the ether insoluble E-i were examined with the other two fractions for free amino acids, polypeptides, and amino N before and after complete hydrolysis; and finally fraction R-1 was examined by paper electrophoresis, two-dimensional polypeptide paper chromatography, and a combination of chromatography and electrophoresis by methods described in the literature. These findings will be discussed in relation to the possible common origin of certain features of these proteins.

1048. PURIFICATION AND PROPERTIES OF TYROSINE ACTIVATING ENZYME. Richard Schweet. Biology Div., California Inst. of Technology, Pasadena.

Tyrosine and tryptophan activating enzymes account for 95% of the amino acid hydroxamide formation of hog pancreas extracts. The activity for tyrosine is 3 times the activity for tryptophan (Schweet, Holley and Allen. Arch. Biochem. In press). The tyrosine activating enzyme has been purified 300-fold from an acetone powder extract. The purification procedure includes adsorption on calcium phosphate gel, precipitation of impurities at pH 5.3 and ammonium sulfate fractionation between 62 and 68% saturation. The purified enzyme catalyzes the formation of tyrosine hydroxamide from hydroxylamine, ATP and tyrosine, and also a tyrosine-dependent exchange of radioactive P-P with ATP. The rate of the exchange reaction is 12 times the hydroxamide formation at all levels of purity, indicating that both reactions are aspects of the same enzymatic activity. The purified enzyme gives no reaction with any other amino acid, using either assay, and no activity is observed in the absence of tyrosine. The absorption spectrum is that of a typical protein without 260 mμ-absorbing material. Both Mg^{++} and K^{+} ions are required for activity. Paper electrophoresis of the enzyme gives a single spot and preliminary studies in the analytical ultracentrifuge show a single peak with $S_{20,w}$ of 4.0.

1049. A SURVEY OF BIOCHEMICAL CHANGES IN POTATOES INITIATED BY Co^{60} IRRADIATION IN THE DECAKILOREP RANGE. Sigmund Schwimmer, William J. Weston* and Rachel U. Makower.* Western Utilization Research Branch, Agricultural Research Service, U. S. Dept. of Agriculture, Albany, Calif.

The values of the following were found to rise above those of unirradiated controls: a) sugars, especially sucrose; b) protein precipitated by 0.6 saturated ammonium sulfate; c) nonenzymatic browning of alcoholic extracts; d) lactic dehydrogenase

activity of soluble protein fraction; e) inorganic phosphate content; f) polyphenol oxidase-induced browning of freshly sliced tuber surfaces as measured by changes in reflectance; g) inhibition of phosphorylase-induced amylose formation by alcoholic extracts. The following were found to decrease: a) rate of chlorophyll formation upon illumination; b) rate of sprout growth; c) apical dominance of sprouts typical of normal untreated potatoes; d) resistance to microbial attack; and e) phosphorylase-priming activity of deionized alcoholic extracts. Little or no consistent effect of irradiation was observed on the following: a) pH of freshly prepared extracts; b) phosphorylase activity of freshly prepared extracts; c) solanine content; and d) inhibition by adenosine triphosphate of the color formed in sliced potato surfaces due to polyphenol oxidase action. Sussman has already shown that the activity of this latter enzyme is not affected at this dosage level (J. Cell. Comp. Physiol., 42:273, 1956). The magnitude and the direction of these effects were found to be dependent upon the previous and postirradiation history of the tubers. Thus tubers eventually recovered mechanisms for sucrose accumulation and for chlorophyll formation; the rate of recovery was dependent upon postirradiation storage temperature.

1050. SULFATE ACTIVATION AND ESTERIFICATION. Harold L. Segal (intr. by R. S. Tipson). Biochemistry Dept., Univ. of Pittsburgh Med. School, Pittsburgh, Pa.

A partial purification of the sulfate esterifying system of liver has been achieved by means of protamine treatment and methanol precipitation. The number of moles of P_i released per mole of sulfate esterified approaches 4, suggesting the participation of more than one mole of ATP in the reaction. On the basis of previous evidence that pyrophosphate is a product of the sulfate activation reaction (Segal. Biochem. et biophys. acta 21:194, 1956), and that a 3', 5'-diphosphate derivative of adenosine is present in active sulfate (Robbins and Lipmann. J. Am. Chem. Soc. 78:2652, 1956) it would appear that the P_i arises from hydrolysis of these compounds by phosphatases found to be present in the enzyme preparations. When the sulfate acceptor is left out of an otherwise complete system active sulfate accumulates which can be assayed in one of several ways, involving charcoal adsorption, paper chromatography or subsequent enzymatic transfer to a sulfate acceptor. The 3 methods gave results which were in good agreement. CoA was found to be a potent inhibitor of sulfate esterification in contrast to a number of other nucleotides and SH compounds tested. Dephospho CoA, produced by hydrolysis of CoA by 3'-nucleotidase, was almost totally inactive in this regard. Variations in Mg^{++} concentration had a pronounced effect on both sulfate activation and esterification. In the latter case a maximum activity was obtained when the Mg^{++} concentration was approximately 1/2 the ATP concentration and a second maximum at very high Mg^{++} concentrations. Tyrosine and several carbohydrates, including amino sugars, were tested and found not to undergo sulfate esterification in this system.

1051. METABOLISM OF D-RIBOSE IN MAN. Stanton Segal, Joseph Foley and James Wyngaarden (intr. by Floyd Daft). Natl. Insts. of Health, Bethesda, Md.

Twenty grams of D-Ribose were infused intravenously into normal subjects over 15-20 min. Blood concentrations of ribose, glucose, inorganic phosphate and pyruvate were determined for 90 min until ribose levels were near zero. Plotting decreasing ribose values on semilogarithmic paper revealed a 2-phase curve, the 2nd phase of which, beginning at levels below 20-30 mg % is a straight line with $t_{½}$ of about 14 min. Glucose was lowered to levels 35-60% of fasting values. The inorganic phosphate and pyruvate were not significantly altered. Renal excretion of ribose was 20 to 30%. Intravenous insulin (0.1 U/kg) accelerated the ribose disappearance rate from blood mainly in the exponential phase. When 5 μc of ribose 1-C^{14} was administered in 20 gm, radioactivity in blood decreased exponentially with t of 65 min. The difference between C^{14} and ribose disappearance from blood was reflected in a gradually increasing ratio of radioactivity to ribose in urine. Of the 36% of the C^{14} dose excreted in 6 hr, about half was ribose; 24% of retained C^{14} was excreted as $C^{14}O_2$ in 6 hr, with maximum radioactivity appearing in 120 min. In 6 hr, 10% of a tracer dose of 5 μc (2 mg) ribose 1-C^{14} in the same subject was excreted renally and 49% of the retained C^{14} as $C^{14}O_2$ with maximum radioactivity in 90 min. These results suggest that 20-gm ribose infusions saturate the relatively slow system metabolizing ribose 1-C^{14} to $C^{14}O_2$ and that ribose is converted to one or more metabolites.

1052. ACTION OF PURIFIED COLLAGENASE ON NEUTRAL SOLUTIONS OF ICHTHYOCOL. Sam Seifter, Paul M. Gallop* and Edward Meilman.* Dept. of Biochemistry, Albert Einstein College of Medicine, Yeshiva Univ., and the Long Island Jewish Hosp., New York City.

Collagenase from Clostridium histolyticum has been purified using several successive procedures including: 1) starch electrophoresis at pH 5.3 in 0.1 M acetate buffer; 2) $(NH_4)_2SO_4$ fractionation between 40 and 60% saturation; and 3) specific adsorption to purified collagen at 0°C, with subsequent digestion of the collagen by the adsorbed enzyme at 30°C and removal of the resulting peptides by dialysis. Purified collagenase requires Ca^{++} for full activity. The mechanism of collagenase action was studied using solutions of a purified ichthyocol in 0.5 M $CaCl_2$, 0.5 M $MgCl_2$, and 0.25 M $M_2Na_2S_2O_3$, each buffered to pH 7.0 with maleate buffer. Experiments were performed at 20°C or lower to minimize gelatinization of the substrate. Enzyme activity with time was followed by measuring changes in viscosity and optical rotation of the solutions. The observed decrease in viscosity, which may be considered as reflecting the appearance of products of small axial ratio, did not give the same rate constant as the observed fall in optical rotation which measures the degree of disruption of the native helical configuration of ichthyocol. These results can be explained by considering the degradation of the collagen to occur in 2 stages: 1) the enzymatic formation of fragments retaining some helical configuration and 2) the thermal severing of the hydrogen bonds of these fragments with the ultimate formation of completely randomized peptidic units.

1053. PHOSPHORYLATION OF ACETOL BY HOMOGENATES OF RAT LIVER. Otto Z. Sellinger* and O. Neal Miller. Dept. of Biochemistry and the Lab. of Nutrition and Metabolism, Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La.

Homogenates of liver, in the presence of ATP and fluoride phosphorylate acetol. Incubation mixtures were inactivated with TCA and the supernatants assayed for the phosphorylated product by measuring the oxidation of $DPNH^+$(340 mμ), in the presence of myogen A (Baranowski. J. Biol. Chem. 180:535, 1949)

in tris buffer, 0.12 M, pH 7.7. Glycerokinase (Bublitz and Kennedy. J. Biol. Chem. 211:951, 1954) failed to phosphorylate acetol as shown by the spectrophotometric assay. Thus a specific kinase seems to be involved. Homogenates that were boiled or dialyzed for 6 hr exhibited no kinase activity. An aliquot of the supernatant from the incubation mixture was treated with crude alkaline phosphatase (30 min, 43°) which dephosphorylated the product and led to no oxidation of $DPNH^+$ in the assay system. Solutions containing the phosphorylated product were freed of nucleotides with charcoal, treated with Dowex 50, neutralized and lyophylized. Paper chromatography (S & S 589, blue ribbon) in t-amyl-alcohol, formic acid, water-35:15:35-demonstrated a spot upon treatment with the Hanes-Isherwood reagent, whose R_f differed from that of inorganic phosphate, propanediol phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate and alpha-glycerophosphate. When the spectrophotometric assay was allowed to go to completion and these solutions were chromatographed as above, a spot appeared whose R_f was identical with that of authentic propanediol phosphate. It therefore appears that the phosphorylation of acetol yields a phosphate ester, which upon reduction is converted to propanediol phosphate, and is therefore presumed to be acetol phosphate.

1054. SURVIVAL OF THE UNDERNOURISHED ORGANISM FOLLOWING TOURNIQUET SHOCK. Kenneth D. Serkes, Morton D. Pareira and Stanley Lang (intr. by Michael Somogyi). Div. of Surgery, Jewish Hosp. of St. Louis, Mo.

The popular concept that mortality is enhanced when surgical trauma is imposed in the presence of nutritional deficiency has been previously tested in acutely and chronically starved rats subjected to various quantitated surgical traumas. No differences in mortality or body weight loss curves were observed between experimental and control groups. The possibility was entertained that undernourished animals might suffer enhanced mortality when subjected to traumas with which significant, acute volemic changes were associated. Tourniquet shock, documented as a situation leading to hypovolemic response was induced in control and starving animals. It was found that a standard tourniquet trauma could be selected which would be sublethal in the control group. This standard trauma when applied to groups of diminishing body weight enhanced mortality. Thus, there was no mortality in the control group, a mortality of 42% in the group starved to 80% of initial body weight and a mortality of 100% in the group starved to 69% of initial body weight. It was then demonstrated that the absolute increments of leg weight gain (representing vascular fluid loss) were roughly identical in the starved and fed groups subjected to tourniquet trauma. When this loss, however, was considered as a percentage of body weight, the loss in the limbs of the starved animals was significantly higher. Enhanced mortality was thus induced in starving animals when subjected to a trauma with which there was associated a disproportionate vascular fluid loss.

1055. IN VITRO CONVERSION OF ADENINE-8-C^{14} TO GUANINE BY HUMAN LEUKOCYTES. Jacob Shapira* and Richard J. Winzler. Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago.

The mode of action of a number of antileukemic agents appears to involve effects on nucleic acid biosynthesis. In the course of studies of nucleic acid metabolism by human leucocytes, the uptake of adenine-8-C^{14} and its conversion of guanine has been studied. The white cells were isolated from patients with chronic lymphatic leukemia (CLL) and chronic myelogenous leukemia (CML) and incubated at 37° in normal human serum under 95% oxygen-5% carbon dioxide for 4 hr with adenine-8-C^{14}. The adenine and guanine present in the nucleic acid of these cells were isolated and the ratio of specific activities of guanine determined. For CLL cells, this ratio was 0.161 ± 0.035 and for CML cells was 0.380 ± 0.046. Chlorambucil, 6-mercaptopurine, Amethopterin and 2,6-diaminopurine at concentrations of 100 μg/ml appeared to have no effect although 2,6-diaminopurine may have had a slight inhibitory effect. It was desired to determine whether this conversion of adenine to guanine was the result of a direct conversion or followed the incorporation of C^{14} into the formate pool and de novo synthesis of guanine. It was necessary to devise a micromethod for the degradation of guanine. This was accomplished by mixing 3-5 μmoles of the isolated guanine with 0.4 N (3 e^-) KMnO in 0.01 N HCl and allowing to stand overnight at room temperature. After decomposition of the excess $KMnO_4$ with oxalic acid, the reaction mixture was subjected to paper ionophoresis at pH 9.6. The guanidine, containing the C_2 of guanine, was completely separated from the urea which contained the C_8 of guanine. These substances were isolated and their specific activities determined. The pooled guanine fractions of the variously inhibited CML cells were degraded and only negligible amounts of activity were found in the guanidine. Serine was isolated from the hydrolyzed protein and was found to contain no detectable activity. Thus, these data support the direct conversion of adenine to guanine.

1056. REACTIVITY OF TRYPTOPHAN ANALOGUES WITH A TRYPTOPHAN-SPECIFIC ENZYME. Nathan Sharon* and Fritz Lipmann. Biochemical Research Lab., Massachusetts General Hosp. and Dept. of Biological Chemistry, Harvard Med. School, Boston.

A recently isolated ATP-linked tryptophan-activating pancreas enzyme was shown also to be reactive with tryptazan (Davie, Koningsberger and Lipmann. Arch. Biochem. 65:21, 1956). In view of the extensive use of tryptophan analogues in biological tests a greater variety of tryptophan analogues has been tested. Such analogues may be divided into two groups: 1) activated like tryptophan and 2) inhibitory to tryptophan activation, but not activated. To 1) belong azatryptophan, 6-fluorotryptophan and tryptazan, which on saturation activate the ATP-PP^{32} exchange at 0.4 to 0.2 the rate of tryptophan. To 2) belong 5- and 6-methyl tryptophan, tryptophan hydroxamate, β-methyl tryptophan and others which inhibit 40 and 100%, at an analogue:substrate ratio of 200:1. It appears significant that only azatryptophan and tryptazan which are enzymatically reactive are incorporated into protein (Pardee et al. Biochem. et biophys. acta 21:406, 1956), while 5-methyl tryptophan inhibits the enzyme as well as growth. This parallelism supports the participation of the amino acid-activating enzymes in protein synthesis.

1057. INCORPORATION OF RADIOACTIVE TRACERS INTO RIBONUCLEOPROTEIN PARTICLES OF RAT LIVER MICROSOMES. Harold T. Shigeura* and Erwin Chargaff. Biochemistry Dept., Columbia Univ., New York City.

As reported previously (Chargaff et al. Nature

178:682, 1956), 2 protein fractions can be distinguished in the deoxycholate-insoluble ribonucleoprotein particles of rat liver microsomes (RNP). After alkaline hydrolysis, slightly more than 50% of the total protein remains insoluble at pH 13.5 (fraction I), the remainder precipitates at pH 6 (fraction II). Since maximal radioactivity is known to be incorporated into RNP within a few minutes after administration of radioleucine (Littlefield et al. J. Biol. Chem. 217:111, 1955), it seemed of interest to investigate the metabolic difference of the 2 protein fractions. At intervals of 5 and 20 min after intravenous injection of DL-leucine-C^{14} into groups of rats, the livers were removed. RNP was isolated and its protein fractions I and II were compared with the deoxycholate-soluble microsomal proteins (DS) and the cytoplasmic supernatant collected after sedimentation of microsomes (Sp). The specific activities were after 5 min: Sp, 32; DS, 125; I, 130; II, 194. After 20 min the corresponding figures were: 50, 228, 82 and 120. The incorporation of P^{32} into the RNA of the different fractions was also investigated. The specific activities of the nucleotides (mixture of 2' and 3' isomers) in Sp were several times greater than in RNP, both 5 and 20 min after radiophosphate injection. After 5 min, the distribution of radioactivity was remarkably uneven in RNP, that of adenylic acid being about 25 times higher than that of cytidylic and guanylic acids; a difference observable, though not as strikingly, in the Sp nucleotides. After 20 min this disproportionateness tends to disappear.

1058. ERYTHROCYTE AND PLATELET THROMBOPLASTIC CELL COMPONENT. George Y. Shinowara. Pathology Dept., Ohio State Univ., Columbus.

Human erythrocytes and platelets contain a lipoprotein which together with a nonclottable globulin in plasma fraction I (Cohn) will quantitatively convert prothrombin into thrombin in the presence of ionic calcium alone (J. Lab. & Clin. Med. 38:11, 1951). The lipoprotein and the globulin have been identified as thromboplastic cell component (TCC) and thromboplastic plasma component (TPC), respectively. The TCC units per million cells range between 1.76 and 4.51 in erythrocytes and 0.24 and 1.25 in platelets (J. Biol. Chem. In press, 1957). In the present study, blood was obtained from normal individuals and sodium citrate was added to aliquots at various times: in silicone experiments, at 0, 15, 30, 60, 90 and 120 min; in glass experiments, at 0, 5, 15, 20 and 30 min. Of the various plasma factors, TPC dropped in concentration most rapidly followed in order by fibrinogen and prothrombin. The antithrombin concentration changes were slight and variable. Since TCC concentrations were exceedingly high in cells and in clots, significant changes could not be found. Therefore, platelet counts and extrastromal hemoglobin were also determined on the various aliquots: It was found that significant concentrations of TCC could be released by platelets during the early phase and by erythrocytes during the later phase of spontaneous coagulation. In another type of experiment, at least 99.3% "pure" erythrocyte and platelet fractions were obtained by differential centrifugation. The effectiveness of minute numbers of erythrocytes as well as of platelets in lowering the calcium times of plasma was demonstrated.

1059. CHANGES IN THE BACTERIAL CELL WALL DURING GROWTH. G. D. Shockman,* J. J. Kolb* and G. Toennies. Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.

Different events follow the logarithmic growth phase (of S. faecalis 9790) upon depletion of an essential amino acid (Growth 13:21, 1949). When valine is depleted logarithmic growth ceases but subsequently the optical density of the culture increases by 50% and dry weight by 30% while total bacterial nitrogen remains unchanged. Cellular amino acids change little, with the exception of a 27% increase in the total amount of lysine. Since lysine is a prominent component of the streptococcal cell wall, other cell wall components, such as rhamnose and glucosamine, were determined, as well as cell wall nitrogen. The fraction of the total cell nitrogen found in the walls (obtained by mechanical disruption) was 13% during the logarithmic growth phase, and 24% after maximum optical density had been attained postlogarithmically. Total cellular nitrogen remained practically unchanged. The 85% increase in cell wall nitrogen was accompanied by similar increases in total rhamnose and glucosamine. Essentially all of the rhamnose was found in the cell wall. The data reveal no change in the composition of the cell wall, but indicate that its amount has nearly doubled. Apparently, under these conditions synthesis of additional cell wall material is the most prominent occurrence in the development of this organism following the end of exponential growth. In this connection, a possible explanation of the phenomenon of spontaneous cellular lysis under conditions of lysine restriction (J. Biol. Chem. 177:831, 1949) will be discussed. (Supported by a contract with the Office of Naval Research.)

1060. SYNTHESIS AND UTILIZATION OF DEOXYRIBOSE-5-PHOSPHATE (DRP) BY NORMAL LIVER AND MALIGNANT HEPATOMA. Carl E. Shonk* and George E. Boxer. Merck Inst. for Therapeutic Research, Rahway, N. J.

The synthesis of DRP from glyceraldehyde-3-P and acetaldehyde is the only enzymatic reaction described for the de novo synthesis of the deoxyribose moiety of DNA. (Racker. J. Biol. Chem. 196:374, 1952). With these substrates and normal liver homogenate (1800 g supernatant), the amount of DRP increases to a peak within about 30 min and is followed by a nearly complete disappearance of formed DRP in 60-90 min. The physiological growth stimulus of liver regeneration increases both synthesis and utilization 2-3 fold. By contrast, in the same fraction of hepatoma homogenate, the synthetic reaction proceeds at a rate 2-3 times faster without any indication of disappearance of DRP even after 3 hr. Addition of normal liver homogenate to hepatoma homogenate completely surpresses the hepatoma pattern of DRP synthesis. With normal liver, the synthesizing capacity is found primarily in the nuclear fraction (600 g precipitate), while the disappearance of DRP occurs only in the high speed (80,000 g) supernatant. With the hepatoma synthetic activity is demonstrable in all centrifugal fractions. The high speed supernatant of the tumor has a low order of activity for DRP disappearance which can be demonstrated only in the absence of substrates for DRP synthesis. In normal liver the disappearance of the deoxyribose moiety occurs aerobically and anaerobically with adenine or guanine, deoxyribosides or deoxyribotides, while deoxyribose and cytidine deoxyribotide are not attacked. This enzymatic activity of the high speed supernatant is lost on dialysis but is restored on addition of boiled supernatant.

1061. C- AND N-TERMINAL AMINO ACIDS OF HUMAN SERUM LIPOPROTEINS. Bernard Shore

(intr. by W. Knowlton Hall). Donner Lab., Univ. of California, Berkeley, and Dept. of Biochemistry, Med. College of Georgia, Augusta.

Determinations of molecular weight, percentage protein, C- (hydrazinolysis) and N- (dinitrofluorobenzene and phenylisothiocyanate methods) terminal amino acids indicate that the 1.093 gm/ml hydrated density, $s^0_{20,w}$ 5.5$_0$ lipoprotein possesses two N-aspartic acid-C-threonine peptide chains, each of approximate molecular weight 95,000; the 1.149 $s^0_{20,w}$ 5.0$_1$ lipoprotein, one N-aspartic acid-C-threonine chain of approximate weight 95,000; the 1.029, S_f 7.9 lipoprotein, at least one, and possibly two, N-glutamic acid-C-serine chains of approximate weight 400,000; the 1.006-1.002 (S_f 20-60) lipoproteins, an N-serine-C-alanine chain of maximum weight 120,000, 1 mole N-terminal glutamic acid/700,000-750,000 gm peptide, 1 mole N-terminal threonine/670,000-800,000 gm peptide, and 1 mole C-terminal serine/250,000-300,000 gm peptide. The 1.029 lipoprotein contained traces of N-terminal aspartic acid, serine and threonine. The proteins of the 1.093 and 1.149 lipoproteins analyzed above were of quantitatively identical amino acid composition and of composition very similar to, but slightly different from, that of the 1.029 and 1.006-1.002 proteins, which seemed identical with each other. The hydrated density, the density of zero sedimentation, was a linear interpolation of values of η_{rel}s for at least three densities. Molecular weights of the 1.093 and 1.149 lipoproteins, 435,000 and 195,000, respectively, were obtained by sedimentation equilibrium; that of the 1.006-1.002 lipoproteins, 50-250 million, was an ultracentrifugal Stokes sphere minimum weight; that of the 1.029 lipoprotein, 3,200,000, a light scattering value of Bjorklund and Katz.

1062. PRECURSORS OF DESOXYRIBOSE IN RAT LIVER STUDIED WITH C^{14}-LABELED GLYCINE. Walton W. Shreeve and Albert Grossman.* Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Two groups of partially hepatectomized Sprague-Dawley white rats were injected daily intraperitoneally for 3 days with glycine-1-C^{14} and glycine-2-C^{14}, respectively. At the end of 3 days the regenerated livers were removed, pooled and treated for isolation of glycogen and nucleic acids. RNA and DNA were separated, DNA hydrolyzed enzymatically and the desoxyribonucleosides separated by ion exchange chromatography. Thymidine was fermented with E. coli (Hoffman and Lampen. J. Biol. Chem. 198:885, 1952), and the split-products of the desoxyribose moiety (ethanol, CO_2, acetic acid) further degraded by chemical means and analyzed for C^{14} content. The relative specific radioactivities of the desoxyribose carbon atoms derived from glycine-1-C^{14} were: C 1 = 19, C 2 = 16, C 3 = 100, C 4 = 6, C 5 = 4. Corresponding values for glycine-2-C^{14} were: C 1 = 60, C 2 = 39, C 3 = 42, C 4 = 100, C 5 = 64. The data do not conform to an origin of desoxyribose from glucose, nor do they indicate a source from ribose, in which patterns of C^{14} distribution are significantly dissimilar (Bernstein. J. Biol. Chem. 205:317, 1953). The data may be construed to support the concept of another pathway of formation of desoxyribose, namely the condensation of acetaldehyde with phosphoglyceraldehyde, as demonstrated in E. coli (Racker. J. Biol. Chem. 196:347, 1952) and in guinea pig liver (McGeown and Malpress. Nature 170:575, 1952). (Research supported by the Atomic Energy Comm.)

1063. PURIFICATION OF FIBRINOGEN. Sidney Shulman and Barbara Blair.* Dept. of Bacteriology and Immunology, Univ. of Buffalo School of Medicine, Buffalo, N. Y.

Bovine fibrinogen has been prepared by centrifugation at 4° of just-thawed plasma (Ware, Guest and Seegers. Arch. Biochem. 13:231, 1947). This material generally has a clottability of 70-80% and is badly contaminated with plasmin and plasminogen. Prothrombin is also present, but not thrombin. These contaminants are detected, respectively, by: 1) incubation at room temperature of a clot formed by thrombin addition; 2) incubation, similarly, but with incorporation of streptokinase and a source of human plasmin; 3) addition of calcium chloride and thromboplastin; 4) incubation at room temperature of a fibrinogen solution. Several variations of each step in the Laki procedure (Laki. Arch. Biochem. 32:317, 1951) were studied and the products were evaluated by all these criteria. It can be shown that the low-temperature, low-salt precipitation can be adjusted to remove plasminogen, while the ammonium sulfate precipitation will remove plasmin and improve the clottability. Other characteristics will also be reported. (Supported in part by a research grant, H1792, from the Natl. Heart Inst., PHS.)

1064. EQUILIBRIUM STUDIES OF THE PHOSPHOGLUCOMUTASE REACTION. J. B. Sidbury, Jr.* and V. A. Najjar. Johns Hopkins Univ. Med. School, Baltimore, Md.

The mechanism of the phosphoglucomutase is visualized as occurring in 2 steps: 1) glucose-1-phosphate + phospho-enzyme ⇌ glucose-1, 6-diphosphate + de-phospho-enzyme, 2) glucose-1, 6-diphosphate + de-phospho-enzyme ⇌ glucose-6-phosphate + phospho-enzyme. K_e for the complete reaction is 17.2. The equilibrium value for the second reaction was determined starting with: 1) glucose-1-phosphate + phospho-enzyme, 2) glucose 6-phosphate + phospho-enzyme, 3) glucose-diphosphate + de-phospho-enzyme. Glucose-1, 6-diphosphate was measured by its stimulation of the phosphoglucomutase reaction, glucose-6-phosphate was measured by the Zwischenferment reaction and de-phospho-enzyme was prepared by enzymatic dephosphorylation with either glucose monophosphate (Science 119:631, 1954). k_2 was determined to be 3.76; since $K = k_1 \times k_2$, k_1 = 4.58. The standard free energy change at 30°C of the respective steps of the reaction was calculated from $\Delta F^\circ = -RT \ln K \cdot \Delta F^\circ$ of step 1) = -914 calories, ΔF° of step 2) = -800 calories, ΔF° of the over-all reaction = -1714 calories. Accepting a ΔF° of hydrolysis of the phosphate bond of glucose-6-phosphate as -3000 calories, then the ΔF° of hydrolysis of glucose-1-phosphate is -4714. Assuming the ΔF° of hydrolysis of the glucosyl-phosphate bond of glucose-1-phosphate and glucose-1, 6-diphosphate to be very nearly equal, it follows that the ΔF° of hydrolysis of the phosphoglucomutase phosphate bond is equivalent to -3914 calories. Thus the ΔF° of the phosphoglucomutase bond would preclude an enol, acyl or guanidine linkage. (Supported by Grant RG-3209 from the Public Health Service.)

1065. PROPERTIES OF INFECTIVE CENTERS INDUCED WITH INFECTIOUS RIBONUCLEIC ACID. A. Siegel, W. Ginoza and S. G. Wildman (intr. by N. S. MacDonald). Botany Dept. and Atomic Energy Project, Univ. of California, Los Angeles.

The early events of infection induced by intact tobacco mosaic virus and the nucleic acid derived therefrom were compared. The method employed was to follow the ultraviolet light sensitivity of infective centers as a function of time after initiation of infection. When intact virus is used as the infecting agent, there is a lag period before the infective center begins

to increase in resistance to ultraviolet light. This lag is approximately 2 hr for the U2 strain and 5 hr for the U1 strain at 20°. On the contrary, there is no lag when nucleic acid is used as the infecting agent; the infective centers begin to increase in ultraviolet light resistance immediately upon initiation. The infective centers initiated with nucleic acid derived from the U1 and U2 strains behave precisely alike, although the strains themselves differ markedly in their in vitro ultraviolet light sensitivity. This information is interpreted to signify that the first event of infection by the intact virus is the release of its nucleic acid from its protein shell. This process is bypassed when the infecting agent is naked nucleic acid. It is further concluded that the nucleic acid of the U1 strain is released with greater difficulty in vivo than that of the U2 strain.

1066. PHOSPHOLIPIDE ANTITHROMBOPLASTIN. M. J. Silver, D. L. Turner, R. R. Holburn, I. R. Schwartz and L. M. Tocantins (intr. by L. A. Kazal). Cardeza Fndn., Jefferson Med. College, Philadelphia, Pa.

Treatment of purified antithromboplastin (Turner and Silver. Fed. Proc. 15:189, 1956) in aqueous solutions with substances generating free radicles and with roentgen rays (Mosher and Limperos, 1950) produced no significant change in the anticoagulant. Treatment with 0.001 M dibenzoyl peroxide or N-bromosuccinimide in chloroform, increased activity from 195 to 280 units. Equimolar N-bromosuccinimide introduced bromine and inactivated the anticoagulant. Heating aqueous dispersions of the anticoagulant to 75° in buffers (pH 3.0-11.0) led to no change in activity, but from pH 11.0 to 12.0 partial inactivation occurred. Some fractions increased in activity to about 280 U/mg after heating with saturated sodium bicarbonate, without change in chemical composition as indicated by N, P, and serine-N. Activity increased further to 320 U/mg by treating the product, after removal of the bicarbonate, by dialysis in 0.5 M potassium dihydrogen phosphate against distilled water. Fractions giving no test for acetal phosphatide retained full anticoagulant activity. Five minutes after intravenous injection (35-80 mg/kg) into dogs of solutions of pork brain phosphatide fractions with high anticoagulant activity by in vitro assay, the blood became incoagulable. Clotting times were still significantly prolonged 24-48 hr afterwards. There was about 5-fold reduction in the generation of plasma thromboplastin. Postinjection plasma and serum lost almost all platelet cofactor activity. Prothrombin was slightly reduced. No significant changes occurred in the levels of fibrinogen, antithrombin, red cells, white cells and platelets. The fractions appeared nontoxic and nonpyrogenic and their water-clear solutions have a stronger anticoagulant activity in vivo than turbid emulsions.

1067. INTRACELLULAR DISTRIBUTION OF RADIOACTIVITY FOLLOWING INTRAVENOUS ADMINISTRATION OF C^{14}-LABELED VITAMIN E. Eric J. Simon, Charlotte S. Gross and Ade T. Milhorat (intr. by Gilbert Stone). Dept. of Medicine, Cornell Univ. Med. College and New York Hosp., New York City.

Mice of the strain in which a mutant for hereditary muscular dystrophy was recently discovered (Michelson et al. Proc. Natl. Acad. Sci. 41:1079, 1955) were injected intravenously with δ-α-tocopheryl-5-methyl-C^{14}-succinate and the free tocopherol obtained by hydrolysis of this labeled ester. The livers were excised after suitable intervals and separated into nuclear, mitochondrial, microsomal and supernatant fractions by differential centrifugation in 0.25 M sucrose. Fractionations were also carried out on livers to which the labeled material was added in vitro. In both in vivo and in vitro experiments 60-80% of the radioactivity of the liver homogenate was recovered in the mitochondrial and microsomal fractions. When the succinate ester was administered the isotope concentration (counts per minute per milligram of total nitrogen) was found to be considerably higher in the microsomes than in the mitochondria. Similar distribution patterns were obtained when mouse liver was homogenized in the presence of either the ester or the free tocopherol. However, the intravenous administration of unesterified vitamin E gave a distribution in which isotope concentration in the mitochondria was approximately twice that in the microsomes. To date no significant difference has been observed between dystrophic animals and their normal litter mates. Evidence indicates that, 24 hr after injection of tocopheryl succinate, the bulk of the radioactive material present in the liver is the unhydrolyzed ester. (Supported by grants from Muscular Dystrophy Associations of America, Inc.)

1068. ERYTHROCYTE FRACTIONATION BY MEANS OF SERIAL OSMOTIC HEMOLYSIS. Ernest R. Simon,* Helen M. Postman* and Yale J. Topper. Natl. Insts. of Health, Bethesda, Md.

$Fe^{59}SO_4$ was administered intravenously to normal human subjects in order to label a circumscribed age group of erythrocytes. At successive intervals thereafter blood samples were delivered into heparin, the erythrocytes washed with unbuffered isotonic saline and then fractionated at room temperature by means of serial osmotic hemolysis in hypotonic saline. The radioactivity per unit hemoglobin was determined on the supernatant derived from each hemolysis and these values were plotted as a function of relative fragility. During the first 2-3 wk following administration of the radioisotope, at a time when only young cells were tagged, the hemolysate obtained from the osmotically most resistant cells had a high specific radioactivity peak, while the fraction obtained from the least resistant cells usually yielded a peak of moderate size. Several weeks later specific radioactivity minima were observed corresponding in position, but not necessarily in magnitude, to the peaks described above. Recurrence of the initial double-peaked pattern was observed about 130 days after labeling. Whereas fractionations performed at 37°C gave results similar to those obtained at room temperature, experiments conducted at 4°C yielded unimpressive maxima and minima. Removal of approximately 80% of the leucocytes prior to fractionation did not alter the results. The implications of these findings will be discussed.

1069. IN VITRO INCORPORATION OF LEUCINE-1-C^{14} INTO THE PROTEIN OF LIVER MITOCHONDRIA. Melvin V. Simpson, John R. McLean,* George I. Cohn* and Ira K. Brandt.* Dept. of Biochemistry, Yale Univ., New Haven, Conn.

The protein of rat skeletal muscle mitochondria has been shown to actively incorporate C^{14}-amino acids in vivo and in vitro (Fed. Proc. 15:312, 1956). Results reported here demonstrate that an in vitro rat liver mitochondrial system possesses similar ability. Mitochondria washed 6 times were incubated in a medium containing Mg^{++}, phosphate, histidine buffer, leucine-1-C^{14}, supernatant fluid (105,000 x g), and an ATP-generating system (phosphorylating mitochondria utilizing succinate or anaerobic systems utilizing creatine phosphate or 3-phosphoglycerate).

Incorporation was inhibited by anaerobiosis or by 2.0×10^{-4} M dinitrophenol in the oxidative system, or by omitting substrate in the anaerobic systems. Omission of supernatant fluid reduced incorporation by 75%. Mitochondria are about 20% as active as microsomes in this system. The following evidence indicates that incorporation into the mitochondrial preparation does not result from possible contamination by the microsomal system: 1) No radioactivity was found in mitochondria isolated from an unlabeled liver homogenate to which had been added a highly labeled, mitochondria-free, microsome suspension prepared by centrifuging at 5000 x g a labeled (in vivo) liver homogenate. 2) High concentrations of ADP or of sucrose in the incubation medium affect the mitochondrial and microsomal systems to a significantly different degree. 3) Addition of ribonuclease completely inhibits the microsomal system whereas the mitochondrial system is stimulated. RNA has consistently been found to be present in the 6-times washed mitochondria, in amounts of about 1 mg/100 mg of protein. (Aided by a grant from Muscular Dystrophy Associations of America, Inc.)

1070. ROLE OF HYDROXYLYSINE IN THE SYNTHESIS OF COLLAGEN. F. Marott Sinex and Donald D. Van Slyke. Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Collagen, the principal protein of connective tissues, contains a unique amino acid, hydroxylysine. The $-CH(OH)-CH_2(NH_2)$ configuration of hydroxylysine is present in the intact protein, since 70-80% of the hydroxylysine molecules of collagen or gelatin were readily destroyed by treatment with alkaline periodate. The hydroxylysine was completely destroyed by periodate if the collagen or gelatin was first treated 16 hr at room temperature with 0.1 N HCl. Experiments with carbon-14-labeled lysine (Sinex and Van Slyke. J. Biol. Chem. 216:245, 1955) showed that dietary lysine serves as the precursor for both the lysine and hydroxylysine of skin collagen. Experiments carried out with DL-hydroxylysine labeled on carbon-atom-6 with either carbon-14 or tritium showed that dietary hydroxylysine is not a direct precursor of the hydroxylysine residues of skin collagen. Animals fed tritium-labeled hydroxylysine or H^3O incorporated a small but measurable abount of tritium into collagen hydroxylysine, presumably via lysine molecules since the lysine of the collagen also contained detectable amounts of tritium. The available data are consistent with the assumption that incorporation of lysine into collagen is accompanied by a change of approximately 1 molecule out of 5 to hydroxylysine. (Research supported by the Atomic Energy Comm.)

1071. INHIBITORY EFFECT OF 2-ETHYL, 5-METHYLBENZIMIDAZOLE ON SYNTHESIS OF PHOSPHOLIPID AND PENTOSENUCLEIC ACID IN CHICKEN RED BLOOD CELLS. S. A. Singal and V. P. Sydenstricker. Depts. of Biochemistry and Internal Medicine, Med. College of Georgia, Augusta.

2-Ethyl, 5-methylbenzimidazole (500 μg), when incubated with 5 ml of washed chicken red blood cells, markedly inhibits the incorporation of radiophosphate in the following fractions: acid-solution phosphorus (inorganic-free), phospholipid and pentosenucleic acid (mononucleotides). Observations at 0, 2, 4, 8 and 12 hr incubation periods indicate that the degree of inhibition varies in the 2 to the 8-12 hr periods as follows: acid-soluble phosphorus, 30-35%; phospholipid, 30-90%; and pentosenucleic acid, 50-90%, respectively. The inhibition observed in the last two fractions cannot be explained entirely upon a possible impairment of oxidative phosphorylation or esterification of phosphorus produced by the inhibitor, as the relative specific activities of these fractions is about 1/5 that found for red blood cells in the absence of the inhibitor. Initial experiments indicate that benzimidazole, 2,3-dimethylbenzimidazole and 2-ethyl, 5-methylbenzimidazole do not markedly affect endogenous oxygen uptake by the red blood cells, although a slow release of inorganic phosphorus is observed.

1072. PHOSPHOROLYSIS OF OLIGORIBONUCLEOTIDES BY POLYRIBONUCLEOTIDE PHOSPHORYLASE. Maxine F. Singer (intr. by H. D. Baernstein). Natl. Insts. of Health, Bethesda, Md.

Polynucleotide phosphorylase catalyzes the reversible synthesis of polyribonucleotides from nucleoside 5'-diphosphates. The phosphorolysis of several oligoribonucleotides by an enzyme from E. coli (Littauer. Fed. Proc. 15:302, 1956) has now been investigated. Oligoribonucleotides of group I were polyadenine compounds containing phosphomonoester groups at C-5', as in the dinucleotide, 5'-phosphoadenosine-3' adenosine-5' phosphate (pApA), the corresponding trinucleotide (pApApA) and tetranucleotide (pApApApA) (Heppel, Ortiz and Ochoa. Science 123: 415, 1956). pApA was not significantly phosphorylized; pApApA and pApApApA were phosphorylized at rates approximately 15 and 30%, respectively, that of AMP-polymer. The comparison of rates was made with reaction mixtures containing 0.8μ moles of adenine residues per milliliter, a concentration which saturates the enzyme with AMP-polymer. However, the enzyme is not saturated with oligoribonucleotide even at concentrations of 5 μmoles adenine residues per milliliter. Exhaustive phosphorolysis of pApApA or pApApApA yields ADP and the inert pApA. When P^{32} was used for the phosphorolysis ADP^{32} of the expected specific activity was isolated. Oligoribonucleotides of group II were mixed adenine-uridine derivatives containing phosphomonoester groups at C-3' (represented as ApUp, ApApUp and ApApApUp) obtained by digestion of A-U polymer with pancreatic ribonuclease and were not phosphorylized at significant rates. Oligoribonucleotides (group III) containing no phosphomonoester moieties (represented as ApA, ApU, ApApA, etc.) were prepared by the treatment of groups I and II with prostatic phosphomonoesterase. The dinucleotides remained inert. ApApA and ApApU were phosphorylized at 5% of the rate observed with AMP-polymer; ApApApA and ApApApU were phosphorylized at 20% of the AMP-polymer rate.

1073. SOME PROPERTIES OF BACTERIOPHAGE ϕX174. Robert L. Sinsheimer. Physics Dept., Iowa State College, Ames.

ϕX174 is one of the group of very small bacterial viruses. Means have been developed for moderately large scale culture of the virus, concentration of the virus from lysates and purification of the virus to obtain an ultracentrifugally homogeneous preparation. The virus has a particle weight of 5,000,000 and contains 35-40% DNA. The purified virus forms stable aggregates; electron micrographs have been obtained of these. Partially purified preparations contain a large, DNA-free, protein component which is thought to be the virus "shell."

1074. PAPER CHROMATOGRAPHY OF BIOLOGICALLY ACTIVE TISSUE CEPHALINS. Vladimir P. Skipski* and Maurice M. Rapport. Sloan-Kettering Inst. for Cancer Research, and Div. of Labs. and Research, New York State Dept. of Health, New York City.

Several components of the "cephalin" type are present in rat lymphosarcoma. This fraction has antigenic activity. We have studied resolution of these components by paper chromatography in an effort to isolate the biologically active substance. Lipids extracted from rat lymphosarcoma were separated into 4 fractions on a silicic acid column (Rapport and Alonzo. Biochemical Problems of Lipids, 1956). Two, eluted with 25% ethanol in hexane and 10% methanol in ethanol, contained all the ninhydrin positive material. These fractions were subjected to further separation by descending paper chromatography on silicic acid impregnated paper, Whatman no. 3MM using various chloroform-methanol mixtures (Lea et al. Biochem. J. 1955). The impregnated paper was extensively washed with water and developing solvent to avoid hydrolysis of acid sensitive plasmalogens. Residual acidity could also be removed by washing with 3% pyridine in developing solvent followed by acetone. After development of the chromatogram, lipid spots were identified by ninhydrin test, OsO_4 vapor, I_2 spray followed by starch dip, Schiff's test, and others. The antigenic activity after elution with methanol was measured by complement fixation. Activity was recovered completely. Three discrete spots were obtained in addition to material remaining at the origin. Antigenic activity was found only in the slowest moving component and at the origin. Studies are proceeding to determine the chemical composition of substances in the individual spots in order to identify them. Resolution in other solvent systems will also be discussed.

1075. EFFECT OF SURFACE-ACTIVE AGENTS ON BIOSYNTHESIS OF RIBOFLAVIN IN ASHBYA GOSSYPII. Curtis G. Smith, Geneva A. Smith and Zoe Papadopoulou (intr. by Jytte Muus). Dept. of Physiology, Mount Holyoke College, South Hadley, Mass.

In the study of the biosynthesis of riboflavin by Ashbya gossypii it has been noted that the use of simple culture media, which are adequate for the growth of the mold, does not result in appreciable yields of riboflavin. Supplements of complex, ill-defined nutrients such as corn steepwater, animal stick liquor, and even peptone, stimulate the production of prodigous quantities of the vitamin (Pridham and Raper. Mycologia 44:452). Adenine has been shown to be a direct precursor of riboflavin in the related mold, Eremothecium ashbyii (McNutt. J. Biol. Chem. 219:365) but the addition of Adenine fails to increase the synthesis in Ashbya. An increase in the flavinogenesis of the mold resulted when a number of surface-active agents such as fatty acid esters of sorbitan and polyoxyethylene, which are produced under the trade names of "Tween," "Span," etc., were added to the growth medium. When Adenine was added in the presence of 2% Tween 80 in a fully-defined synthetic growth medium (containing asparagine, inositol, biotin, thiamin, magnesium sulfate and glucose) the amount of riboflavin produced is more than twice that previously reported. Further experiments afford evidence to support the thesis that it is the surface-activity rather than a direct nutritional role which accounts for the observed effects of surfactants.

1076. PHOSPHORYL-S-COENZYME A: AN INTERMEDIATE IN SUCCINATE ACTIVATION. Roberts A. Smith,* Irma F. Frank* and I. C. Gunsalus. Div. of Biochemistry, Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.

Succinyl-S-Coenzyme A formation according to reaction 1,

$$ATP + CoASH + Succinate \rightleftarrows Succinyl\text{-}S\text{-}CoA + ADP + iP \quad (1)$$

is catalyzed by extracts of aerobically grown Escherichia coli. In common with the spinach P-enzyme of Kaufman (J. Biol. Chem. 216:153, 1955) the reaction is ATP linked. Fractionation of the E. coli extracts has demonstrated 2 enzyme reactions and an intermediate CoA derivative, i.e.,

Coenzyme A Thiolkinase: $ATP + CoASH \rightleftarrows ADP + CoA\text{-}S\text{-}PO_3H_2$ (2)

Phosphoryl CoA Transferase: $CoA\text{-}S\text{-}PO_3H_2 + Succinate \rightleftarrows Succinyl\text{-}SCoA + iP$ (3)

Coenzyme A Thiolkinase was measured by reaction 4:

$$phosphopyruvate + DPNH + CoASH + ATP \rightleftarrows lactate + DPN + ATP + CoA\text{-}S\text{-}PO_3H_2 \quad (4)$$

by following DPNH oxidation in the presence of added lactic dehydrogenase and phosphopyruvatekinase. The equilibrium of reaction 2, catalyzed by 100-fold purified thiolkinase, containing 240 units/mg P (1 U = .01 O.D. change/min 340 mμ) as calculated from ADP formation and SH disappearance was K = ca. 0.035 indicating phosphoryl CoA to be about 2000 calories more reactive than ATP. Phosphoryl-CoA-transferase, purified about 10-fold (12 Kaufman units/mg P), has not been completely freed of CoA thiolkinase. The catalysis of reaction 3 can be measured from the equivalent formation of succinylhydroxamate and inorganic phosphate with phosphoryl-CoA as substrate in the presence of NH_2OH. Phosphoryl-CoA is stable to 3 min at 100° at pH 7.3, thus permitting accumulation of this substrate by reaction 4 and removal of the enzymes. The mechanism of the phosphorylation reaction and α-ketoglutarate oxidation will be discussed in the light of these experiments.

1077. ISOLATION OF AN EXTRACELLULAR PROTEIN FROM PENICILLIN-INHIBITED ESCHERICHIA COLI CELLS. Robert C. Smith* and S. B. Binkley. Dept. of Biochemistry, Univ. of Illinois School of Medicine, Chicago.

It has previously been reported (Binkley. Arch. Biochem. 61:84, 1956) that an extracellular protein accumulates when E. coli 9723 is grown in a medium containing 10 units of penicillin per milliliter. This protein has been isolated and compared to the ribonucleoprotein of both normal and penicillin-inhibited cells. It was isolated from growth cultures which had been clarified by centrifugation. The pH of the supernatant was lowered until the protein precipitated out at its isoelectric point. This crude preparation was purified using a DEAE-SF column and eluting with buffer of increasing concentration. The ribonucleoprotein of both control and penicillin-inhibited cells was isolated by extraction of the lyophilized cells with 0.14 M NaCl at 5°C and chromatographed on a DEAE-SF column. Both paper and moving boundary electrophoretic studies were made on these purified proteins. The amino acids of these proteins were determined by paper chromatography of their hydrolysates. All 3 of the proteins show the same electrophoretic mobilities and amino acid composition. The amino acid composition of these proteins is very similar to that found in the cell wall of E. coli H (Salton. Biochem. et biophys. acta 10:512, 1953), differing only in the amino acids serine and threonine. The results indicate that in this organism penicillin may be interfering with the synthesis of a cell wall protein.

1078. SODIUM TRANSPORT SYSTEM OF ISOLATED FROG SKIN. Fred M. Snell and O. R. McIntyre

(intr. by F. Lee Rodkey). Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.

Some measurements were made to study the influence of hydrogen ion and carbon dioxide concentrations on the sodium transport system of the isolated abdominal skin of Rana pipiens. The net flux rate of sodium is determined by measuring the current flow across the skin with the skin potential shunted to zero. Two such cells allow symmetrical portions of the skin to be studied simultaneously, one serving as a control. Variations in the bathing solutions are made on both sides of the skin. Utilizing 6 mM phosphate buffered Ringer's the results are in essential agreement with those of Schoffeniels (Arch. internat. physiol. 63:513, 1955) in that the net flux rate decreases with decreasing pH. Almost complete inhibition of sodium transport is obtained at pH 6.2, although this effect is reversible. The results with HCO_3^-:5%CO_2 buffered Ringer's indicate that on passing from a phosphate buffered Ringer's to the HCO_3^-:5%CO_2 buffer at the same pH there is a significant transient decrease in the flux rate. On returning again to the phosphate buffer there is a marked transient increase in the flux before steady values are again obtained at the initial rate. These observations suggest a rapid diffusion of CO_2 to and from the site of the transport system, followed by the slower readjustments of HCO_3^- in altering the pH. Diamox in concentrations up to 10 mM appears to be without effect. (Supported by a grant from the Public Health Service.)

1079. UTILIZATION OF SIMPLE NITROGENOUS COMPOUNDS BY THE GROWING INFANT. Selma E. Snyderman, L. Emmett Holt, Jr., Joseph Dancis,* M. Earl Balis and Audrey Boyer.* Dept. of Pediatrics, New York Univ. College of Medicine, and Sloan-Kettering Inst. Div. of Cornell Univ. Med. College, New York City.

In the course of studies designed to evaluate the minimal protein requirement of infants, the protein intake of 3 infants fed on cow's milk was gradually reduced by decreasing the amount of milk while the caloric intake was kept constant by the addition of fat and carbohydrate. Weight gain ceased when the protein intake was reduced to less than 6% of the calories (1.95 gm protein/kg). In order to find out whether the limiting factor at this point was an essential amino acid or some unessential nitrogenous compound supplements of glycine and of urea were added. These supplements made it possible to reduce the protein intake to 4% of the calories (1.27 gm/kg) without impairing weight gain although such developed if protein were lowered beyond this. Normal performance at this low level of protein intake was continued for several weeks; weight gain continued and there was good nitrogen retention in the 2 subjects in whom balance studies were carried out. One study in which N^{15} urea was fed revealed that the urea was being incorporated into plasma protein and into hemoglobin in considerable amounts. It is apparent that the limiting factor in a low protein diet of cow's milk is "unessential" rather than "essential" nitrogen. Such unessential forms of nitrogen can contribute significantly to protein anabolism in the human.

1080. INFLUENCE OF ATP ON CALCIFICATION IN VITRO. Albert Edward Sobel and Martin Burger.* Dept. of Biochemistry, Jewish Hosp. of Brooklyn, Brooklyn, N. Y.

Rachitic bone sections, treated for 2 hr at 37°C with 1 mM ATP containing 25 to 75 mM of Ca^{++} at pH 7.1 to 7.3, calcified in vitro in inorganic media at Ca x P products of 20 (4 mg % Ca, 5 mg % P) and 10 (2 mg % Ca, 5 mg % P). This calcification included both the hypertrophic cartilage and the bare osteoid. Control sections, treated with Ca^{++}, or fresh sections, under the same conditions, did not exhibit calcification in vitro. These control sections calcified at Ca x P products of 40 (8 mg % Ca, 5 mg % P), or higher. This calcification did not extend into the bare osteoid but was limited to the hypertrophic cartilage. When ATP was placed in the medium employed for calcification in vitro, inhibition of calcification in vitro was obtained even at a Ca x P product of 50 (10 mg % Ca, 5 mg % P). Theoretically the formation of a solid from an undersaturated solution should require some form of energy for concentrating the ions forming the solid, while in a saturated or supersaturated solution, nuclei would be required, the formation of which may be aided by appropriate forms of energy. Whether preliminary treatment with ATP enhances the activity of the calcifying mechanism by providing energy for one or both of the aforementioned postulates, or by direct formation of nuclei not involving energy is the subject of further investigation. (These studies were supported in part by: 1) a grant from the Natl. Inst. of Dental Research, PHS, 2) a contract between the Office of Naval Research, and the Jewish Hosp. of Brooklyn NR 180 025, and 3) funds provided under contract AF18 (600)-1274 with the USAF School of Aviation Medicine, Randolph Field, Texas.)

1081. SEDIMENTATION AND VISCOSITY STUDIES OF BOVINE FIBRINOGEN SOLUTIONS AT VARIOUS IONIC STRENGTHS. Raymond Sowinski,* Lubomyra Oharenko* and Virgil L. Koenig. Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.

Bovine fibrinogen (BF) was dissolved in solutions of NaCl having concentrations of 0.1, 0.2, 0.3, and 0.5 M. Solubility of a given weight of BF varied from 1% to 3.6% depending upon the ionic strength of the solvent. Sedimentation determinations were made on at least 8 concentrations of BF in the NaCl solution at each ionic strength studied. The Spinco analytical ultracentrifuge was used. The straight lines for the relationship of sedimentation constant (S_{20}) to concentration (c) at each ionic strength were obtained by the method of least squares. The intercepts, representing S_{20} at zero concentration in water, varied from 7.34 to 7.48 Svedberg units. The slopes for these straight lines varied from -0.31 (0.1 M NaCl) to -0.77 (0.5 M NaCl). Viscosity studies were carried out on at least 7 concentrations of BF in NaCl at ionic strengths of 0.1, 0.2, 0.3, and 0.5 M. Determinations were made at 20°C using an Ostwald capillary viscometer. Weight intrinsic viscosities ($[\eta]$) were determined both from the slopes of the straight lines resulting from the $1/\eta_r$ vs c and the $\ln\eta_r$ vs c relationships. These relationships were obtained by the method of least squares. It was found that $[\eta]$ did not vary significantly with the ionic strength of the solution. The differences in the slopes of the lines of regression for S_{20} on c at different ionic strengths were interpreted, therefore, as being dependent upon the charge of the BF molecule in the centrifugal field.

1082. POSITIONS OF DISULFIDE BRIDGES IN RIBONUCLEASE. Darrel H. Spackman,* Stanford Moore and William H. Stein. Rockefeller Inst. for Med. Research, New York City.

Ribonuclease in 2 M guanidine·HCl has been treated with trypsin and then with chymotrypsin in the presence of N-ethylmaleimide (Sanger et al. Biochem. J. 60:541, 1955). The ninhydrin color value of the protein solution was increased to 3 times the

starting value. The major part of the guanidine was removed by brief dialysis (Craig and King. J. Am. Chem. Soc. 77:6620, 1955) and the resulting mixture was chromatographed on Dowex 50-X2. Peptides containing -S-S- groups were detected in the effluent fractions by a modification of the method of Kassel and Brand (J. Biol. Chem. 125:115, 1938). The materials present in the principal cystine-positive peaks were desalted on NH_4-Dowex 50 and oxidized by performic acid. When the products of oxidation were chromatographed on Dowex 50-X2, as before, the cysteic acid peptides were found in peaks preceding the position of the cystine-free components of the mixture. After quantitative determination of their amino acid composition, the individual cysteic acid peptides could be placed in the partial structural formula of ribonuclease (Hirs et al. J. Biol. Chem. 221:151, 1956). In this manner, the two major -S-S- positive zones provided evidence for -S-S- bridges between the 1st and the 6th half-cystine residues [isolation of ($CySO_3H$, Asp-NH_2, Glu-NH_2, $MetSO_2$) and ($CySO_3H$, Asp,Ileu,Thr,Ser,Arg)] and the 4th and 5th half-cystine residues [isolation of (Asp-NH_2, $CySO_3H$,Ala,Val,Lys) and ($CySO_3H$,Asp-NH_2,Asp-NH_2,Glu-NH_2,Gly,Thr,Tyr)]. Separation of the peptides arising from the other two -S-S-bridges is being undertaken. The above results are in agreement with the findings of Anfinsen and Ryle on this subject (personal communication).

1083. METABOLIC SEQUENCE OF EVENTS IN LIVER AND DIAPHRAGM OF RATS ACUTELY DEPRIVED OF INSULIN. Robert Spiro (intr. by A. B. Hastings). Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.

The sequence of events leading to the characteristic metabolic picture of long-standing diabetes in liver slice and isolated diaphragm and the role of insulin lack per se has been studied. C-14 labeled glucose and fructose were used as substrates. Alloxan diabetic rats, restored to normal metabolic function by 2-wk insulin treatment, were acutely made insulin deficient by discontinuing its administration. The animals were killed at intervals up to 96 hr measured from the 1st injection of crystalline insulin. Blood sugar rose to diabetic levels in 6 hr. By 20 hr liver glycogen was depleted, liver lipide was doubled, yet lipogenesis by the liver was drastically reduced. Liver glucose-6-phosphatase was increased 3-fold in 34 hr. There was a similar rise in conversion of fructose to glucose with a concomitant decline of fructose to glycogen. At 14 hr, glucose phosphorylation by liver was reduced 40%. Similarly, glucose uptake by diaphragm fell rapidly to 50% at 10 hr. This was followed by a slow decline in both these functions so that even after 48 hr values characteristic of the chronic diabetic state had not been reached. However, such values were attained by 96 hr. It would appear that the changes in glucose uptake by diaphragm and phosphorylation by liver take place in two stages.

1084. ENZYMATIC SYNTHESIS OF SPHINGOMYELIN. Michael Sribney* and Eugene P. Kennedy. Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

The enzymatic synthesis of lecithin has been shown to proceed by a reaction between cytidine diphosphate choline and a D-α,β-diglyceride (Kennedy and Weiss. J. Biol. Chem. 222:193, 1956). Since sphingomyelin also contains a phosphorylcholine moiety in a monophosphate diester linkage, it might be expected by analogy that the enzymatic synthesis of sphingomyelin would involve a reaction between cytidine diphosphate choline and N-acyl sphingosine (ceramide). An enzyme has now been found in chicken liver which catalyzes a reaction between cytidine diphosphate choline and an "active sphingolipide" obtained by partial hydrolysis of a pure cerebroside (phrenosine) in acetic-sulfuric acid (Klenk. Ztschr. physiol. Chem. 153:74, 1926). The product of this enzymatic reaction is a phospholipide with properties resembling those of sphingomyelin. The enzyme requires manganese ions for maximal activity and it is necessary to emulsify the "active sphingolipide" in a surface-active agent (Tween-20). The enzyme is specific for cytidine diphosphate choline. The structure of the "active sphingolipide" and the nature of the enzymatically synthesized phospholipide have not yet been established with certainty.

1085. CO-FACTOR REQUIREMENTS IN THE CONVERSION OF 2-KETO-3-DEOXY-7-PHOSPHOGLUCOHEPTONIC ACID (KDPH) TO 5-DEHYDROQUINIC ACID (DHQ). P. R. Srinivasan* and David B. Sprinson. Dept. of Biochemistry, Columbia Univ., New York City.

Evidence has been adduced to indicate that the path from glucose to DHQ proceeds by way of 1) D-erythrose-4-phosphate and phosphoenolpyruvate, 2) KDPH, and 3) a still incompletely identified intermediate which is formed from DKPH in crude extracts of Escherichia coli containing 10^{-2} molar Versene (cf. Rothschild, these proceedings). In fractions obtained by treatment of the crude extracts with protamine and $(NH_4)_2SO_4$, followed by dialysis against glycylglycine buffer, the synthesis of DHQ from KDPH is inhibited by 10^{-3} molar Versene and is restored by the addition of Co^{++}. Treatment of these extracts with charcoal prevents the further conversion of KDPH. The activity is restored by the addition of Co^{++} plus DPN (or DPNH). The requirement for pyridine nucleotides has been confirmed with Neurospora DPNase. The intermediate formed in crude extracts treated with Versene is converted by the above fractionated extracts to DHQ in the presence of Co^{++}. After treatment with charcoal, DPN or DPNH is required to convert this intermediate to DHQ.

1086. ACTIVATION OF A BACTERIAL DPNase BY OXIDATION-REDUCTION SYSTEMS. E. R. Stadtman. Natl. Heart Inst., Bethesda, Md.

Cell-free extracts of Clostridium propionicum catalyze the decomposition of DPN to adenosine, nicotinamide mononucleoside and 2 equivalents of orthophosphate. By means of protamine, isoelectric and ammonium sulfate precipitation, the extracts have been separated into 2 protein fractions, A and B, both of which are essential for DPNase activity. In addition, DPN decomposition also requires the presence of a suitable electron transport system; i.e. a pyridine nucleotide dehydrogenase together with its appropriate substrate, and an electron acceptor such as flavin mononucleotide (FMN), triphenyltetrazolium, methylene blue or 2,6 dichlorophenol indophenol. The pyridine nucleotide dehydrogenase system may be replaced with a catalytic amount of DPNH or with xanthine oxidase together with its substrate, hypoxanthine. When xanthine oxidase is the electron donor system, only partial requirements for FMN and fraction A are observed. In the complete system, DPNase is almost completely inhibited by 10^{-2} M arsenate, 10^{-2} M inorganic pyrophosphate, and by 10^{-3} M concentrations of various purine and pyrimidine nucleoside di- and tri-phosphates. 10^{-2} M orthophosphate inhibits 40%. ATP and inorganic pyrophosphate are not hydrolyzed by the complete system. Orthophosphate is released from adenylic

acid and nicotinamide mononucleotide when incubated with the complete system containing xanthine oxidase and hypoxanthine. Although the reactions are carried out in tubes gassed with helium, a need for the electron transport systems to generate hydrogen peroxide is suggested by the observation that, when pyridine nucleotide reducing systems are used, DPNase activity is completely suppressed by catalase.

1087. PHOSPHATE ESTERIFICATION COUPLED TO GLYCINE REDUCTION IN ENZYME PREPARATIONS OF CLOSTRIDIUM STICKLANDII. Thressa C. Stadtman (intr. by J. C. Keresztesy). Natl. Heart Inst., Bethesda, Md.

It has been shown with enzyme preparations of Clostridium sticklandii (J. Am. Chem. Soc. 78:2020, 1956) that the reductive deamination of glycine by 1,3-dimercaptopropanol leads to the formation of ATP. The stoichiometry of the over-all process is:

$$\text{glycine} + R(SH)_2 + Pi + ADP \rightarrow \text{acetate} + NH_3 + R\text{-}SS + ATP$$

The enzyme system catalyzing the over-all reaction has now been separated into two distinct protein fractions. With many of these fractions it has been possible to achieve combinations that show up to 2 moles of phosphate esterified (10 min P) per mole of acetate formed. Examination of these reaction mixtures for other glycine metabolites revealed the presence of a compound (or compounds) in the neutral volatile fraction containing both carbon atoms of glycine. The material formed from 2-C^{14} or 1-C^{14}-labeled glycine is detected following its conversion to $C^{14}O_2$ by various oxidizing agents under conditions where substances such as ethanol are less extensively oxidized. This neutral-volatile material does not react with carbonyl reagents. Balance experiments and further chemical characterization studies are in progress.

1088. INACTIVATION OF TMV-RNA WITH FORMALDEHYDE. Matthys Staehelin (intr. by H. Fraenkel-Conrat). Virus Lab., Univ. of California, Berkeley.

The possibility that the nucleic acid (RNA) might be involved in the inactivation of tobacco mosaic virus (TMV) by formaldehyde (Ross and Stanley. J. Gen. Physiol. 22:165, 1938) was suggested by the uptake of formaldehyde and the spectral changes occurring in RNA after reaction with formaldehyde (Fraenkel-Conrat. Biochim. et biophys. acta 15:307, 1954). A direct study of the reaction was made possible by the finding of infectivity in RNA isolated from TMV (Fraenkel-Conrat. J. Am. Chem. Soc. 78:882, 1956). TMV-RNA was found to be very sensitive to formaldehyde treatment, the concentrations necessary for inactivation being less than one-hundredth of that of intact TMV. Experiments with formaldehyde C^{14} show that two different reactions take place. The greater part of the formaldehyde is bound in a reversible form and lost on dialysis, while a smaller part is more firmly bound and cannot be removed by dialysis. The relative amount of the firmly bound fraction increases with the time of the reaction. The absolute amount of formaldehyde bound to the nucleic acid under conditions which lead to partial inactivation is one molecule of formaldehyde for several hundred nucleotides. Indications for an increase in molecular weight suggest that cross linking of RNA molecules occurs after reaction with formaldehyde. The amount of formaldehyde taken up by nucleic acids from different sources varies considerably, TMV-RNA being much more reactive than RNA from yeast and undenatured DNA showing almost no uptake.

1089. OXIDATION OF CHOLESTEROL AND TRIHYDROXYCOPROSTANE BY IN VITRO SYSTEMS. Ezra Staple and Michael W. Whitehouse (intr. by C. S. Rose). Dept. of Biochemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.

The cell-free system from C3H mice livers described by Anfinsen and Horning (J. Am. Chem. Soc. 75:1511, 1953; Fed. Proc. 13:212, 1954) for the side chain oxidation of cholesterol has been shown capable of oxidizing the terminal methyl groups of 3α, 7α, 12α trihydroxycoprostane (THC) to yield carbon dioxide. The terminal carbon atoms (26 and 27) of THC were labeled with C^{14} by synthesis from triformylcholyl chloride and di-isopropyl-1,3-C^{14} cadium and subsequent reduction of the 24 keto compound. 24-Keto THC and 24-hydroxy THC were not significantly oxidized by the system. Both mitochondria and supernatant fractions from liver homogenates were required for the oxidation of THC, the microsomal fraction being inhibitory. The animal source was critical, C3H mice livers being much more active than those of other strains tested (Runner-Jax, Swiss Hygienic) or rats. The role of THC and the related 3α, 7α, 12α trihidroxycoprostan-27-oic acid as intermediates in bile acid formation from cholesterol will be discussed.

1090. CONVERSION OF CIRCULATING GLUCOSE TO CO_2 IN NORMAL, HYPOPHYSECTOMIZED AND ADRENALECTOMIZED DOGS. R. Steele, J. S. Wall,* N. Altszuler* and R. C. deBodo. Biology Dept., Brookhaven Natl. Lab., Upton, N. Y. and Dept. of Pharmacology, New York Univ. College of Medicine, New York City.

The pattern of $C^{14}O_2$ excretion following intravenous injection of $HC^{14}O_3^-$ was found to be similar in normal, adrenalectomized and hypophysectomized dogs. Knowing this pattern it was possible to calculate (Steele. J. Biochem. 60:447, 1955) conversion of circulating glucose to CO_2 by tissues of resting male unanesthetized dogs in the postabsorbtive state during experiments (Steele et al. Am. J. Physiol. 187:16, 1956) in which the body glucose pool was tagged with C^{14} to measure the rate of uptake of glucose by the tissues. In each hormonal state about one-half of the glucose carbon taken up was converted rapidly to CO_2 and about one-sixth of the total CO_2 expired arose from combustion of circulating glucose by the tissues. Circulating glucose combustion represents only one-third of total carbohydrate combustion calculated from nonprotein R.Q.'s of normal dogs (deBodo et al. Recent Progress in Hormone Research VIII, 511, 1953) under similar conditions. Neither adrenalectomy nor hypophysectomy influences metabolic pathways in such a way as to change the fraction of the glucose entering cells which proceeds to CO_2. In male adrenalectomized dogs total CO_2 production and glucose uptake were in the normal male range; in male hypophysectomized dogs both CO_2 production and glucose uptake were considerably reduced. Notwithstanding absence of growth hormone, lowered thyroid hormone secretion and probable lowered insulin secretion in the hypophysectomized dog, circulating glucose was burned at the same rate relative to other CO_2-producing substrates.

1091. STRUCTURE OF B. SUBTILIS α-AMYLASE. Eric A. Stein (intr. by Aaron Bodansky). Dept. of Biochemistry, Univ. of Washington, Seattle.

Recrystallized α-amylases from human, hog, bacterial and fungal origin have been shown to contain at least one gram-atom of very firmly bound calcium per mole (Vallee, Stein and Fischer). Various reports have indicated that these enzymes undergo irreversible

deactivation in the presence of metal binding agents; however, when purified (Fischer and Stein. Arch. sci. (Geneva) 7:131, 1954) in the presence of diisopropyl-phosphofluoridate (DFP), they can retain full activity even after prolonged treatment with these agents. It is believed that only degraded or denatured amylases (as opposed to intact proteins) are susceptible to deactivation by chelating agents. DFP protects the amylases from a proteolytic degradation that otherwise usually occurs during purification and that cannot be detected by activity measurements. Sedimentation and diffusion studies have been performed on native, protease-free, crystalline B. subtilis α-amylase. This enzyme contains, in addition to calcium, one-half gram-atom of zinc per mole. It was found that after treatment with metal binding agents, the sedimentation constant of the enzyme falls from 6 to 4 Svedberg units, without significant change in the viscosity of the protein solution. This transformation, which is reversible, does not involve the bound calcium but only zinc. The following reaction is proposed: $[\text{Ca-prot.}]\text{-Zn-}[\text{Ca-prot.}] \rightleftharpoons 2\,[\text{Ca-prot.}] + Zn^{+2}$. The molecular weight and frictional ratio of the monomer and dimer have been determined. Both forms are active; the dimer, which is the naturally occurring form, is the only one which has been crystallized.

1092. TOXIC EFFECTS OF HIGH LEVELS OF DELTA-4-CHOLESTENONE IN THE DIET. Daniel Steinberg, Donald S. Fredrickson,* and Grant W. Liddle.* Natl. Heart Inst., Bethesda, Md.

Previous studies have shown that feeding of Δ^4-cholestenone to rats strikingly inhibits incorporation of C^{14}-acetate into cholesterol in the liver and lowers serum cholesterol levels. At a level of 0.1% in a synthetic diet the compound is well tolerated but at a level of 1% it is highly toxic. The rats fail to gain weight and there is about a 20% mortality in 8 wk. The only outstanding post-mortem finding is a 4-7 fold increase in adrenal weight, which appears to be a true hypertrophy. The concentration of cholesterol in these adrenals is very low and on a sodium-free diet the treated animals show decreased salt-retaining capacity. The addition of 1% cholesterol to the cholestenone-containing diet permits the animals to gain at a normal rate but does not prevent adrenal hypertrophy. Cholestenone is known to be converted to dihydrocholesterol in the body and the latter compound appears to accumulate in the livers of rats fed high dosages of Δ^4-cholestenone. The feeding of equivalent doses of dihydrocholesterol itself does not duplicate the marked toxic effects of Δ^4-cholestenone with respect to weight gain. Over an 8-wk period there is, however, a 2-fold adrenal enlargement. The toxicity of Δ^4-cholestenone is apparently not attributable to conversion to dihydrocholesterol in the intestine but the possibility that such conversion subsequent to absorption is contributory cannot be ruled out. In contrast with these results in rats, guinea pigs fed 1.5% Δ^4-cholestenone tolerate the drug well and show no decrease in urinary steroid excretion.

1093. IN VITRO SYNTHESIS OF CYSTEINE FROM SERINE AND OF LACTIC ACID FROM CYSTEINE AND SERINE BY TUMOR OR LIVER TISSUE OF TUMOR-BEARING MICE. Jakob A. Stekol, Sidney Weiss and Ethel I. Anderson.* Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.

We previously reported (Am. Chem. Soc. 122nd Meeting, Sept. 1956) that homogenates of tumors of mice or rats do not efficiently synthesize cysteine from serine and homocysteine. Cleavage of L-cystathionine to cysteine by tumors or livers, however, was efficient and about equal in extent. In the present study we investigated the activity of serine dehydrase (as one of the enzymes involved in cysteine synthesis from serine) and of cystine and cysteine desulfhydrase (enzymes responsible for possible destruction of synthesized cysteine) in tumors and host livers. Tracer techniques were used. Compared to that in the liver, the activity of serine dehydrase in tumors is low, and the supplementation with pyridoxal phosphate, DPNH, and adenylic acid (reported cofactors of L-serine dehydrase) did not raise the activity in tumors to that observed in the liver. No activity of cystine or cysteine desulfhydrase was found in unfortified tumor homogenates; addition of DPNH and pyridoxal phosphate raised the activity of cystine desulfhydrase to that of unfortified liver homogenates. DPNH was required for cystine desulfhydrase activity apparently for prior reduction of cystine to cysteine, since only pyridoxal phosphate was required for activation of cysteine desulfhydrase in tumors. Compared to uniformly labeled glucose, cystine and cysteine, under aerobic or anerobic conditions, are excellent sources of lactic acid in unfortified livers or in tumor homogenates fortified with pyridoxal phosphate and DPNH. Under anerobic conditions, unfortified liver homogenates synthesize as much lactic acid from L-serine as they do from glucose. L-Cystathionine is not cleaved to pyruvic acid and homocysteine by either tumor or liver. It appears that serine dehydrase is one of the limiting factors in cysteine synthesis from serine in tumors, and that pyridoxal phosphate is the limiting cofactor in cysteine desulfhydrase activity in tumors. (Aided by grants from Natl. Cancer Inst., PHS, and Atomic Energy Comm.)

1094. SEPARATION OF DENATURED DNA FROM NATIVE DNA. Vincent L. Stevens* and Edward L. Duggan. Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

Specimens of DNA denatured by heating or by brief exposure to acid or alkali are precipitated at low lead ion concentration. Similar specimens which have not received these treatments do not precipitate under the same condition. The titrimetric studies of DNA have shown that ionic history of a DNA sample defines the number of groups available for hydrogen ion combination in the interval of pH 8 - 4. Previous work (Fed. Proc. 14:205, 1955) indicated that lead, zinc or other divalent ions precipitate DNA at pH 4 with hydrogen ion release. The protons appearing (1/12P) exceeded a reasonable number for terminal phosphate. Protons could also be released from amino groups by base pair formation; alternatively, protons could dissociate from enol groups as a lead enolate formed. This work used Pb/P ratios of 45-1. At lower lead concentration (7/1 ratio), this ion has been effective in the formation of precipitates of heat-treated DNA. Untreated DNA does not form this product. Mixtures of heated and unheated DNA yield the expected absorbance following lead addition and centrifugation, on the basis that only the unheated DNA remains soluble. For 2-min heating periods, the DNA samples exhibit a critical temperature for "deformation" such that an 87° sample will not show precipitation with lead ion while a 90° sample will precipitate. Heating in the presence of lead ion lowers these temperatures approximately 30°. The conversion of DNA to a form which precipitates with lead ion is also accomplished by 1-min exposure (at room temperature) to acid or alkali. Any of these precipitates will redissolve in disodium Versenate.

1095. FACTORS AFFECTING INTERACTION OF THIAMINE PYROPHOSPHATE AND YEAST CARBOXYLASE. H. B. Stewart (intr. by F. C. Heagy). Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.

Extracted yeast carboxylase preparations are inhibited by anions (Cl^-, Br^-, SO_4^{--}, PO_4^{---}, pyruvate$^-$, lactate$^-$, tartrate^{--}, citrate^{---}, etc.). The degree of inhibition is related to the pK of the acid from which the anion arises. Anion inhibition is dependent upon the pH, being virtually negligible on the alkaline side of the pH optimum and increasing as the pH is lowered. Titration of thiamine pyrophosphate (TPP) indicates that the inhibition, which has neither the characteristics of competitive nor non-competitive inhibition, is maximal when only one phosphate group of TPP is dissociated. Dialysis of carboxylase preparations against cysteine in citrate buffers at various pH's has shown that increasing hydrogen ion or anion (Cl^-) concentrations produce enzyme inactivation. This can be largely reversed by the addition of Mg^{++} and TPP. Anion inhibition is specifically antagonised by TPP. Conversely, carboxylase preparations, in the absence of salts other than citrate buffer, show a pronounced stimulation of activity in the presence of high concentrations of TPP. This stimulation is largely prevented by the addition of neopyrithiamine pyrophosphate in a ratio of 20:1 with TPP. It is proposed that the effects described are a consequence of a competition between TPP^{---} and added anions for cationic sites on the apoenzyme.

1096. ENZYME SUBSTRATE INTERACTION IN A FLAVOPROTEIN SYSTEM. Elizabeth Steyn-Parvé* and Helmut Beinert. Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

When a saturated fatty acyl CoA is added to one of the fatty acyl dehydrogenases, the enzyme (E) appears stabilized at an intermediate oxidation state by strong binding of substrate (S) (Beinert, Biochim. Biophys. Acta, 20:588, 1956). Some properties of this ES-complex are under investigation. The spectral changes which occur during E-S interaction are not reversed by dialysis or repeated salt precipitation. Enzymatic activity is however unchanged. At pH 2 the flavin is liberated from protein but release of a CoA derivative could so far not be detected. Attempts will be made to decide in what form S is bound, whether as saturated or unsaturated CoA derivative or as acyl residue separated from CoA. Special attention was given to the green butyryldehydrogenase. When this enzyme is reduced by dithionite or substrate its color disappears slowly. On reoxidation the enzyme is no longer green but yellow and has increased activity (~80%). After versene dialysis, which does not change activity or spectra, neither the green nor the yellow form has significant amounts of Cu. The ES-complex of the green form has the same spectrum as that of the yellow form. Since the green form is less active than the yellow one and since the green color is abolished by S, it appears likely that the green color is due to a complex of the enzyme with a substance which competes with S for the active site. The shift of the flavin bands to shorter wavelengths in the green form implicates the flavin in such a complex.

1097. EXCRETION OF AN ESTRIOL-LIKE METABOLITE FOLLOWING ADMINISTRATION OF 1,3, 5(10),16-ESTRATETRAEN-3-OL. Benjamin F. Stimmel. Rees-Stealy Clinic Research Fndn., San Diego, Calif.

Following administration of M. N. Huffman's synthetic estratetraenol, men excreted a butanol extractable substance in the urine, which after β-glucuronidase hydrolysis and partial purification, was chromogenic in the Kober test for the natural estrogens (estratetraenol likewise is chromogenic in the Kober test). The steroid moiety of the urinary metabolite, unlike estratetraenol, was hydrophylic on distribution between benzene and 1 N NaOH. It was eluted from a column of alumina in the estriol fraction of our chromatographic procedure (estratetraenol appeared in the estrone fraction). The absorption spectrum (320-500 mμ) with 90% H_2SO_4 was similar to that of estriol. Its hemiphthalate, like estriol hemiphthalate, was hydrophylic on distribution between ether and aqueous buffer pH 7.3, and the partition coefficients obtained with the Craig countercurrent apparatus were in good agreement. The urinary metabolite was chromogenic in the Bachman sodium phenol sulfonate test which is specific for estriol. However, it exhibited a somewhat different absorption curve (max. 520 mμ) for that of estriol (max. 540 mμ). Colorimetric estimation of the urinary metabolite in the 3-day post-treatment urine, expressed as estriol, was equivalent to 10-20% of the administered estratetraenol (5-10 mg). A much smaller amount of unchanged estratetraenol (except for conjugation) was colorimetrically demonstrable in neutral steroid extracts of the post-treatment urine.

1098. ABSENCE OF REDUCED PYRIDINE NUCLEOTIDE (D(T)PNH) CHANGES DURING PHOTOSYNTHESIS IN CHLORELLA. Bernard L. Strehler. Univ. of Chicago, Chicago, Ill.

Various lines of evidence have been taken to suggest the photochemical participation of D(T)PNH in photosynthesis. In order to assay [D(T)PNH] in vivo during phosynthesis, we have developed an ultra-sensitive photofluorometer which will detect reduced nucleotide fluorescence in small amounts of intact Chlorella. The algae were excited to fluorece with a very dim 365 mμ source and the selectively filtered nucleotide fluorescence was monitored with a quantum counter. Appropriate filters also permitted cross illumination with red photosynthesis-inducing light. When Chlorella cells are illuminated in the presence of 5% CO_2-air, there is no measurable increase in fluorescence attributable to D(T)PNH. By contrast, when the light is extinguished, there is a marked and rapid decrease in [D(T)PNH] followed by a dark recovery to the prior steady state. Removal of CO_2 produces a striking increase in the [D(T)PNH] as indicated by fluorescence. In the absence of CO_2, there is a slight increase in the amount of reduced nucleotide during illumination and, in the dark, a simple return to the steady state. D(T)PNH was identified as the preponderant fluorescent compound measured and was assayed with the luminous bacterial enzyme system ([DPNH] = 10^{-4} - 10^{-5} M). These results are in conflict with the prevalent views on the role of pyridine nucleotides in photosynthesis. They strongly suggest that 1) nicotinic coenzymes are not reduced photovhemically, 2) light does not affect nucleotide level directly, 3) nicotinic coenzymes are not normally limiting in photosynthesis, 4) DPNH is in dark equilibrium with some CO_2 derivative (PGA?), and 5) some intermediate or reductant other than D(T)PNH limits photosynthesis.

1099. OXIDATION OF CHOLINE TO BETAINE ALDEHYDE BY RAT LIVER MITOCHONDRIA. D. R. Strength. Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.

Betaine reineckate is soluble in 0.2% Na_3PO_4 and insoluble in 0.3 N HCl solutions and choline reineckate is quantitatively crystallized from the basic solution. The reineckate of the product of the incubation of

choline with mitochondria possesses the same solubility characteristics as choline reineckate. The treatment of aliquots of the incubation mixture with Ag_2O resulted in the formation of betaine which was recovered as betaine reineckate by crystallization from acidic medium. Measurement of oxygen uptake and determination of choline and betaine as their respective reineckates before and after treatment with Ag_2O permitted the calculation of betaine aldehyde present in the incubation mixture. Incubation of 10 mg of choline with mitochondria from 400 mg of rat liver for 2 hr produced approximately 4 mg of betaine aldehyde. Recovery of reineckates insoluble in Na_3PO_4 solution constituted 97-101%. A dry mixture of choline and betaine aldehyde reineckates is stable for several months. The 2,4-dinitrophenylhydrazone of betaine aldehyde was formed following the decomposition of the mixed reineckate with $AgNO_3$, precipitation of excess silver ion as AgCl and the addition of 2,4-dinitrophenylhydrazine. The phenylhydrazone was recrystallized from ethanol-water mixture, m.p. 121°. The phenylhydrazone in alkaline solution exhibits λ max 438 and was used as a standard for the development of a colorimetric assay for betaine aldehyde. The assay is effective for 15 to 100 γ of betaine aldehyde with an accuracy of ±2% at 30 γ of betaine aldehyde. Blanks of deproteinized tissue, nonincubated choline, or betaine are approximately the same as the reagent blank.

1100. OXIDATION OF L-α-AMINO ACIDS BY CHICKEN LIVER MICROSOMES. Jacob Struck* and Irwin W. Sizer. Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.

Enzyme preparations from chicken liver have been found to carry out the oxidative deamination of certain L-amino acids. Examination of cell fractions prepared in 0.25 M sucrose by conventional methods of differential centrifugation reveals that the enzyme activity is mainly associated with the microsome fraction. Products of the reaction have been identified as ammonia and the α-keto acid analogue of the amino acid substrate. The reaction may conveniently be followed by measurement of the keto acid produced. Routine assays have utilized as substrate L-leucine, which is the most rapidly oxidized of the substrates tested. Other substrates readily oxidized are the L-isomers of isoleucine, α-amino butyric acid, methionine, tryptophane and tyrosine. Valine, alanine and the basic amino acids are oxidized only very slowly if at all. Glycine, glutamic acid, aspartic acid, proline and hydroxyproline are not oxidized. Oxygen is required for the reaction to proceed. Extensive dialysis of microsome suspensions does not diminish the activity and the addition of various coenzymes does not produce an increase in enzyme activity. Versene, p-chloromercuribenzoate, arsenite, azide, inorganic pyrophosphate and ammonium ions do not inhibit the reaction. Cyanide causes partial inhibition (73%) at a relatively high concentration (5×10^{-3} M). Cytochrome c, ferricyanide, and 2,6-dichlorophenol-indophenol do not serve as hydrogen acceptors with microsome preparations. While the activity can be obtained from chicken liver acetone powders, the enzyme system appears to be firmly bound to insoluble particles. Attempts to solubilize and further purify the enzyme by conventional methods have been unsuccessful to date.

1101. ENZYMIC SYNTHESIS OF OLEIC AND PALMITIC ACIDS BY HIGHER PLANTS. P. K. Stumpf and George A. Barber.* Dept. of Agricultural Biochemistry, Univ. of California, Berkeley.

Particles from avocado fruit mesocarp incorporate as much as 30% of C^{14}-labeled acetate into long chain fatty acids in 2 hr at 30°, anaerobically, with ATP, CoA and Mn^{++} as the essential cofactors. Other nucleotides and metal ions are inactive. No externally added reducing systems were necessary. The newly synthesized acids do not occur as free acids but are bound as esters of xanthophyll and glycerol. The nature of the products was established by 1) reversed phase paper chromatography and 2) gas chromatography. Only palmitic and oleic acids had considerable incorporation of C^{14} derived from acetate. Lauric, myristic and stearic acids were essentially non-radioactive. De novo synthesis rather than a simple exchange occurred. Thus, when radioactive fatty acids formed from acetate-1-C^{14} were submitted to a Schmidt-azide decarboxylation, about 1/8 of the total radioactivity was released as $C^{14}O_2$ indicating a uniform distribution of activity throughout the odd carbons of the long chain fatty acids. The mechanism for oleic acid synthesis is being further investigated. (Supported in part by a grant from the Natl. Science Fndn.)

1102. SPECIES VARIATION IN PICOLINATE AND QUINOLINATE FORMATION FROM 3-HYDROXYANTHRANILATE BY LIVER EXTRACTS. R. J. Suhadolnik,* C. O. Stevens,* L. M. Henderson and L. V. Hankes. Dept. of Chemistry and Chemical Engineering, Univ. of Illinois, Urbana, and Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Carboxyl labeled 3-hydroxyanthranilate (3-OHAA) was incubated with liver extracts from 10 animal species. All extracts oxidized this substrate at approximately the same rate, giving rise to the unstable intermediate (Compound I) in yields as high as 90%. In certain species, notably the rat, mouse and pig, low decarboxylase activity resulted in the release of less than 13% of the C^{14} as CO_2. The corresponding values for other species were as follows: hamster, 19%; dog, 26%; guinea pig, 31%; rabbit, 34%; pigeon, 37%; beef, 49%; and cat, 86%. With few exceptions, the quinolinic acid present accounted for the remainder of the C^{14}. 3-OHAA labeled with tritium on the benzene nucleus was prepared from 2-nitro-3-methoxybenzoic acid which had been tritiated by pile neutron bombardment in the presence of lithium carbonate. Following incubation of this tritium-labeled substrate with cat and rat liver extracts, carrier picolinic acid (PA) and quinolicic acid (QA) were added and these 2 products isolated from each incubation mixture. The percentage recoveries of the tritium were 60.6 in PA and 16.6 in QA for the cat and 7.4 in PA and 72.0 in QA for the rat. These results demonstrate that PA is the major product of 3-OHAA oxidation by cat liver preparations. The results of in vivo experiments will also be reported.

1103. A NEW AND DIFFERENTIATING TEST FOR GALACTURONIC ACID. M. S. Sullivan and Suad A. Al-Kassab.* Georgetown Univ., Washington, D.C.

In chemical studies of plants and animals it became necessary to devise tests differentiating galactose and its congeners from glucose and its congeners. By various procedures we have succeeded in differentiating the various congeners-galactosamine vs. glucosamine, galacturonic acid vs. glucuronic acid, etc. For the galacturonic acid we are outlining a color reaction. Without giving essential details and in large-scale work Felix Ehrlich Ber. 65B, 352, 1932 mentioned a precipitation reaction for galacturonic acid—a brick-red precipitate with freshly filtered basic lead acetate. Following Ehrlich's directions

more or less, we obtained the brick-red precipitate but could not get it into solution without loss of color. Many modifications of the reaction were made and finally a soluble color reaction was devised not given by other sugars or derivatives. This color reaction is as follows: a dilute solution of galacturonic acid treated with 0.1 ml of a 1% alpha naphthol in 95% alcohol and then with 5 ml of 40% freshly filtered basic lead acetate gives a red solution readable in the Bausch and Lomb spectronic 20. Glucuronic acid under similar treatment gives a colorless solution. Application of the reaction in various fields will be detailed.

1104. FREE AMINO ACIDS IN LUNGS OF NORMAL AND INFLUENZA VIRUS INFECTED MICE. Donald F. Summers and C. A. Johnson (intr. by Olaf Bergeim). Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago.

Groups of 40 (CFw) male mice, averaging 31-34 gm, were infected intranasally with a virulent (PR8) strain of influenza A virus. Identical groups were inoculated with heat-inactivated virus. At the end of 4 days the animals were anesthetized, exsanguinated, the lungs excised, and quickly frozen with dry ice. Small aliquots of the tissues were removed for infectivity tests; the larger portions were weighed, suspended in hot dilute acetic acid, and boiled for 4 minutes to inhibit enzyme action. The suspensions were homogenized, diluted to 5 times the original tissue weight, and the proteins removed by centrifugation and precipitation with tungstic acid. Amino acids were determined microbiologically in the neutralized protein-free filtrates and the levels in normal mouse lungs, expressed in μg/gm moist tissue, were as follows: arginine 135, cystine 42, glycine 488, histidine 39, isoleucine 52, leucine 263, lysine 81, methionine 39, phenylalanine 67, proline 14, serine 265, threonine 151, tryptophan 13, tyrosine 44, and valine 176. These amino acid levels were not significantly altered in the lungs of virus infected mice. In embryonated eggs group assays of the infected lungs gave virus titers of 10^{-5} ID_{50}/ml. The lungs of the control mice appeared normal, and no evidence of virus could be demonstrated by these infectivity tests. Comparison of levels of free amino acids in lungs with those of other tissues will be discussed. (Supported by the Natl. Fndn. for Infantile Paralysis.)

1105. CONTROL OF PHOSPHORYLATIVE RATE IN WASHED RAT LIVER MITOCHONDRIA. Marjorie A. Swanson, Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.

It has previously been reported from this laboratory that the phosphorylating rate of washed rat liver mitochondria in a medium composed almost entirely of organic substances remains practically constant up to complete utilization of phosphate and acceptor. This finding, which is contrary to most of those reported in the literature, has been further investigated. All tests have been made in the presence of a 1 AMP : 2 phosphate ratio, since changes in this ratio have been found to induce changes in the rate. The optimum pH varies with the substrate. For succinate it occurs at pH 7.0, for glutamate 7.5, for proline and glutamine 7.7, for alpha-keto-glutarate the peak rate extends from pH 6.9 - 7.6. If the medium is not heavily buffered, the pH rises as phosphorylation proceeds with any of these substrates, thereby changing the rate as the optimum is approached and passed. Combinations of substrates usually yield a higher rate than either substrate alone. Addition of ATP usually enhances the phosphorylating rate; related triphosphonucleotides sometimes but not always depress the rate. A number of other factors have also been investigated and will be discussed.

1106. METABOLISM OF STEROID HORMONES BY EXTRA-HEPATIC TISSUES AND TARGET ORGANS. Max L. Sweat, Bernard I. Grosser,* Charles C. Nabors* and H. E. Swim.* Depts. of Obstetrics and Gynecology and of Anatomy, Univ. of Utah College of Medicine, Salt Lake City, and Department of Microbiology, Western Reserve University School of Medicine, Cleveland, Ohio.

Approximately 10,000 C.P.M. (1.6-3.0 μg) of radioactive hydrocortisone, progesterone, estradiol, and estrone were each incubated in vitro (pH 7.3) with a variety of extra-hepatic tissue preparations. Steroid conversions in these tissues appear to be extensive. Both progesterone and estradiol are converted to other steroids by human endometrial tissue. Estrone has not been demonstrated to be metabolized by human endometrium. Human skin converts progesterone to at least 1 compound of lower polarity. Lung tissue (bovine) converts progesterone to at least 2 compounds of greater polarity. Human tissue culture fibroblasts (U12) metabolize progesterone to at least 9 different steroids. Pregnane-3,20-dione; 4-pregnene-20beta-ol-3-one; 4-pregnene-20 alpha, 01-3-one have been identified. Two 20-hydroxy steroids have been partially identified. The fibroblasts convert hydrocortisone to at least 6 different steroids; 4-pregnene 11beta,17alpha,20beta,21 tetrol-3-one and 4-pregnene 17alpha,20beta,21 triol-3,11-dione have been identified. Neither human serum nor the incubation medium employed in the tissue culture cells have been observed to effect steroid conversions over a 5-day incubation period under sterile conditions. The steroids were extracted and identified by the isotope methods of Berliner (Fed. Proc. 15:219, 1956; Anal. Chem. 28:1608, 1956). (Supported by grants from the PHS.)

1107. RELATION OF ISOLEUCINE REQUIREMENTS TO THE DIETARY NITROGEN LEVEL. Marian E. Swendseid, Nancy A. Fischer,* Ruth M. Jackson* and Stewart G. Tuttle.* Univ. of California, Dept. of Home Economics, and VA Ctr., Los Angeles.

The amount of isoleucine required to maintain nitrogen balance was determined for 4 young adults fed semi-synthetic diets containing 6.5-7.0 gm nitrogen/day. The essential L-amino acids were given as a mixture in the amounts present in 18.75 gm whole egg protein. Glycine was the source of supplementary nitrogen. Experimental procedures were similar to those of previous studies (Swendseid, and Dunn. J. Nutrition 58:507, 1956) and isoleucine requirements were found to be in the same range. The same subjects were then fed a semi-synthetic diet containing 12.8 gm nitrogen/day. When the mixture of essential L-amino acids was again given in the amounts present in 18.75 gm whole egg protein with supplementary nitrogen being provided by glycine, all subjects went into negative nitrogen equilibrium. However, the subjects were maintained in balance when whole egg was substituted for the essential amino acid mixture and supplementary nitrogen was furnished by approximately equal amounts of glycine and diammonium citrate. When the amount of whole egg was reduced to furnish the previously determined isoleucine requirement for the subject with the essential amino acids except isoleucine being added to maintain constant levels, and glycine and diammonium citrate again providing supplementary nitrogen, all subjects were in negative nitrogen balance. When the isoleucine amount was doubled, 3 subjects went into

balance, but one did not attain a state of nitrogen equilibrium.

1108. ENZYMATIC STEPS IN THE FORMYLATION OF TETRAHYDROFOLIC ACID BY FORMIMINOGLUTAMIC ACID IN LIVER. Herbert Tabor. Natl. Insts. of Health, Bethesda, Md.

Previous studies from several laboratories have reported the enzymatic conversion of histidine and urocanic acid to formiminoglutamic acid (FIGLU). We have recently shown (Tabor and Rabinowitz. J. Am. Chem. Soc. 78:5705, 1956) that further metabolism of FIGLU in rabbit liver involves the following steps: FIGLU + tetrahydrofolic acid (THF) $\xrightarrow{I}$ 5-formimino-THF $\xrightarrow{II}$ 5,10-methenyl-THF $\xrightarrow{III}$ 10-formyl-THF. 5,10-Methenyl-THF (anhydro-citrovorum factor) can be differentiated from the other compounds by its absorption maximum at 355 mμ; the appearance or disappearance of this absorption affords a convenient enzyme assay. The 3 enzymatic steps and the spectrophotometric assay are comparable to reactions previously reported (Rabinowitz and Pricer. Ibid. 78:5702, 1956) for the degradation of formiminoglycine by Clostridium cylindrosporum extracts. The liver enzymes have been found in several species; considerable variation has been noted in the relative amounts of the 3 activities in different species. Enzyme I (FIGLU transferase) has been purified 500-fold from hog liver acetone powder by ammonium sulfate and isoelectric precipitations. It has been largely separated (>95%) from enzyme II (cyclodeaminase), and is free of enzyme III (cyclohydrolase). Similar steps, followed by incubation (0°) at pH 9.2, have been used to purify (500-fold) enzyme II (cyclodeaminase); this preparation is essentially free of enzymes I and III. Enzyme III (cyclohydrolase) has been purified from rabbit liver acetone powder, since very little activity was found in hog liver acetone powder extracts. This activity was obtained, completely free of enzymes I and II, in a fraction precipitated between 1.2 and 2 M ammonium sulfate.

1109. URINARY EXCRETION OF 8-HYDROXYQUINALDIC ACID AFTER INGESTION OF XANTHURENIC ACID. Hitoshi Takahashi* and J. M. Price. Cancer Research Hosp., Univ. of Wisconsin Med. School, Madison.

Previous studies revealed that oral administration of kynurenic acid led to the urinary excretion of quinaldic acid by man and the rat. It now appears that xanthurenic acid may be metabolized in a similar manner. Five rats were each given 33.7 mg of xanthurenic acid daily for 7 days. The pooled urine was subjected to ion-exchange chromatography to isolate the 8-hydroxyquinaldic acid. The recrystallized material (32 mg) melted at 214.5° alone and at 214° when mixed with authentic 8-hydroxyquinaldic acid (m.p. 214°). The spectrum of the natural product was identical with that of synthetic 8-hydroxyquinaldic acid, and the natural and synthetic compounds chromatographed on paper or in ion-exchange columns in an identical manner. The natural and synthetic compounds were decarboxylated to give identical products which could not be distinguished from authentic 8-hydroxyquinoline. Quantitative ion-exchange chromatography revealed that normal rat urine contained less than 0.05 μM of free 8-hydroxyquinaldic acid. After ingestion of 164 μM of xanthurenic acid rats excreted a total of about 3 μM of free 8-hydroxyquinaldic acid in 48 hr. After ingestion of 820 μM of xanthurenic acid by man it was impossible to isolate crystals of 8-hydroxyquinaldic acid, but paper chromatography of the concentrated urine fractions revealed a spot with an ultraviolet spectrum and R_F values identical with 8-hydroxyquinaldic acid. It would appear that dehydroxylation of 4-hydroxyquinolines may be a general type of biochemical reaction.

1110. OXYGENATION OF BOVINE HEMOGLOBIN-DIELECTRIC STUDIES. Shiro Takashima* and Rufus Lumry. Univ. of Minnesota School of Chemistry, Minneapolis.

Appreciable dielectric-increment changes of horse hemoglobin solutions during oxygenation (J. Am. Chem. Soc. 78:591, 1956) provide evidence for conformational involvement of globin in the reaction. Further impedance studies on this protein have demonstrated that dielectric increment and dielectric relaxation time parallel each other through a series of minima and maxima as the equilibrium oxygen pressure is increased. Similar studies have now been carried out using bovine hemoglobin. At 15° increment and relaxation time pass through a maximum (0.37; 15.9×10^{-8} sec), a minimum (0.17; 10.0×10^{-8} sec), a second maximum (0.31; 15.9×10^{-8} sec) to reach final values (0.22; 13.2×10^{-8} sec) for oxyhemoglobin. Cole and Cole plots fit the data within experimental error and we may assume that their distribution function is appropriate for our relaxation times. In general the distribution is broad. The dielectric phenomena are spread over a wider range of oxygen partial pressures than for horse hemoglobin in keeping with different oxygen affinities. The similarity of the 2 proteins which we find provides preliminary evidence for the generality of the oxygen-produced dielectric phenomena. Our data suggest that appreciable alteration in the shape and symmetry or charge distribution occurs during oxygenation thus perhaps demonstrating a connection long suspected between conformation and function in mammalian hemoglobins. Preliminary studies of dielectric effects produced by carbon monoxide uptake at 25° show less exotic behavior. Minima and maxima, if present, are very greatly reduced. (Supported by the Office of Naval Research.)

1111. EFFECTS OF "C-1" DONORS ON EXPERIMENTAL PORPHYRIA. Ellen L. Talman* and Robert F. Labbe (intr. by W. R. Todd). Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.

Previous work in this laboratory has established impaired purine biosynthesis as part of the syndrome of experimental porphyria. This phenomenon of increased porphyrin formation accompanied by decreased purine formation has been explained on the basis of work by Shemin et al. showing that δ-aminolevulinic acid (ALA) serves as a precursor of both porphyrins and purines. Thus the reciprocal relationship between porphyrin and purine biosynthesis may reflect a block in Shemin's succinate-glycine cycle at the point where ALA undergoes oxidative deamination. Since ALA participates in purine biosynthesis by contributing its δ-carbon atom to the "C-1" pool, it seems likely that the purine deficiency constitutes only one manifestation of a deficit in one-carbon fragments. Therefore, dietary supplementation with nutrients contributing single-carbon units by pathways not involving ALA should alleviate the porphyria while "C-1" donors metabolized via ALA should aggravate the porphyria. Accordingly, several physiological "C-1" donors have been administered to embryonated eggs, via the yolk sac, simultaneously with a porphyria-producing drug (allylisopropylacetamide) and the porphyrin excretions of the chick

embryos followed at 48-hr intervals for 6 days. As predicted, glycine and ALA increase the porphyrin output of porphyric embryos; histidine diminishes porphyrin excretion throughout the experiment, while methionine fails to reduce porphyrin output until the later stages of the experiment. Serine, although an excellent "C-1" donor, yields a molecule of glycine for each one-carbon fragment produced so that treatment of porphyric embryos with this amino acid increases porphyrin output despite its contribution of a "C-1" unit.

1112. MODELS WITH THE ACTIVITY OF THE ENZYME HYDROGENASE. N. Tamiya,* S. L. Miller* and D. Rittenberg. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.

The enzyme hydrogenase, which activates molecular hydrogen and catalyzes the exchange reaction between H_2 and D_2O, is of interest because of the simplicity of its substrate. In the course of studies on the nature and mechanism of action of the enzyme hydrogenase, we have investigated model compounds, both inorganic and organic, which catalyze this exchange. Platinum which is known to be an active hydrogenation catalyst is not a good model for hydrogenase because the hydrogen is activated by a homolytic split. Hydrogenase, however, splits the hydrogen to give an enzyme hydride and a proton. We know this because when the exchange between H_2 and D_2O is catalyzed by platinum the first species to appear in the gas phase is D_2, while with hydrogenase as the catalyst the first species to appear is HD. (Krasna and Ritternberg. J. Am. Chem. Soc. 76:3015, 1954). Homogenous catalysis of the exchange reaction by strong bases such as OH^- or NH_2^- in liquid ammonia is known. We find catalytic activity by various complexes of Cu^{++}, Ag^+, Hg^+; the acetates of Cd^{++}, Zn^{++}, and Mg^{++} and the uroporphyrin complexes of Cu^{++}, Fe^{+++} and Ni^{++}. The insoluble MoS_2 is an active catalyst for the activation of hydrogen. MoS_2 will catalyze the reduction of methylene blue and of benzyl viologen by hydrogen. Like hydrogenase the mechanism of the activation appears to involve a heterolytic split of the hydrogen to a proton and a hydride ion. On the basis of these results, we suggest that the active site of the enzyme consists of a Lewis acid which accepts the hydride and a basic group which accepts the H^+. The characteristics of the H^- acceptor and the role of the protein will be discussed.

1113. OXIDATIVE METABOLISM OF ACETOBACTER PEROXIDANS. Stuart W. Tanenbaum (intr. by B. F. Erlanger). Dept. of Microbiology, Columbia Univ., New York City.

Additional compounds tested as substrates in the continuing examination of oxidative pathways in A. peroxidans were acetoin, diacetyl, tartronic acid and ethylene glycol. Only the last-named was attacked. Cell-free extracts exhibit TPNH and DPNH oxidases, and pyruvic carboxylase. It has not yet been possible to obtain a cell-free system which oxidizes acetate; and citrate, isocitrate, cis-aconitate and α-ketoglutarate are not oxidized under any experimental conditions thus far tried. As cells are aged in the cold permeability changes occur, and the pH must gradually be lowered to maintain acetate and succinate oxidations. A study of the effect of fluoroacetate, difluoroacetate, fluoroethanol and trifluoroethanol on substrate and coenzyme oxidations is in progress. Besides inhibiting acetate and succinate oxidations in whole cells, fluoroacetate stops the initial dehydrogenation of malate and of pyruvate. Fluoroethanol itself is oxidized slowly and inhibits ethanol oxidation by virtue of its prior conversion to a substance at the fluoroacetaldehyde level. The inhibitory action of the fluoro analogues is also related to cell age and thus to cell permeability, as best seen with difluoroacetate and trifluoroethanol, which preferentially stop oxidations by aged cells. A. perosidans grown in the presence of radioactive calcium carbonate and unlabeled ethanol incorporated less than 1% of the activity. During 100-min oxidation of acetate-2-C^{14} by resting cells, all of the protein amino acids became labeled. Little or no activity was found in the "pool" components. It is concluded that the process of oxidative assimilation may be more complex than is allowed for by the simple stoichiometric equations derivable from manometric data.

1114. BREAKDOWN OF PARTICULATES FROM RAT LIVER. Harold Tarver and Asher Korner.* Dept. of Physiological Chemistry, Univ. of California School of Medicine, Berkeley.

The proteins in the livers of rats were labeled by feeding them either yeast-S^{35} or Rhodospirillum rubrum-C^{14}. Two or 3 days later the animals were sacrificed, their livers were homogenized in a sucrose-containing medium and fractionated into nuclei, mitochondria and microsomes by centrifugation. The release of the label from the several fractions on incubation in suitable media at pH 7 was then studied under different conditions, under oxygen, under nitrogen, with DNP (10^{-4} M), and with ATP plus creatine phosphate (CP). Most experiments were carried out with carrier amino acids in the medium to minimize reincorporation of labeled amino acid. In agreement with the results from tissue slices (Simpson; Steinberg and Vaughan) a greater release of labeled amino acid was generally found under oxygen as compared with nitrogen or in the presence of DNP. In the presence of ATP plus CP, there was actually, with the microsomes and nuclei, a decrease in label in the medium during the first hour of incubation; with the mitochondria just an inhibition of release. During the period from the 2nd to the 4th hr of incubation, with all preparations under all conditions tried, there was an increase in the amount of label in the medium. When incubations were carried out at pH 5.3 there was a great acceleration in the release of label from all 3 preparations. In the absence of carrier amino acids the release of label was in all cases greatly decreased.

1115. NEISSERIA GONORRHOEAE ENDOTOXIN. Henry Tauber and Warfield Garson.* Venereal Disease Exptl. Lab., PHS, School of Public Health, Univ. of North Carolina, Chapel Hill.

It has been observed that washed gonococcus cells dried over sodium hydroxide in vacuo dissolve readily in 0.05 N sodium hydroxide at pH 13.00. Under these conditions presumably all of the cell-bound endotoxin is freed. Other investigators have previously used a much less alkaline pH for the gradual extraction of cell-bound endotoxin. Under those conditions only a small portion of the endotoxin is obtained. When our lysate is adjusted to pH 7.40, freed of cell debris, dialyzed, and the supernatant adjusted to pH 4.80 with acetic acid, a precipitate forms which consists mainly of nucleoprotein. The precipitate contains the endotoxin. This confirms the observation of earlier investigators. Toxicity, however, in our hands varies with different strains of gonococcus. Quantitative data will be presented concerning the DNA content, the toxicity of different fractions as oposed to the DNA content, and the toxicity of the dry cell suspensions.

The gonococcus was grown in an atmosphere of air using a new liquid medium which was found to be suitable for the large-scale cultivation of the organism.

1116. AN UNIDENTIFIED IODINE COMPOUND FORMED BY CELL-FREE FRACTIONS OF THYROID, SALIVARY AND OTHER TISSUES. Alvin Taurog, W. Tong* and I. L. Chaikoff.* Dept. of Physiology, Univ. of California, Berkeley.

A particulate fraction from sheep thyroid tissue (mitochondria plus microsomes), incubated in vitro with I^{131}-iodide, produces an iodinated protein containing I^{131}-monoiodotyrosine. In addition, 2 unidentified I^{131}-compounds are formed. One of these, referred to as Unknown 2, was the major iodinated product formed by particulate fractions of the following non-thyroid tissues incubated with I^{131}-iodide: submaxillary, lactating mammary and spleen. Liver was very slightly active, kidney completely inactive. Submaxillary was the most active tissue tested; its soluble fraction was more active than its particulate fraction. The soluble fraction of all other tissues was inactive. Formation of Unkown 2 was inhibited by anaerobic conditions, and by 10^{-3} M thiouracil, thiocyanate, thiosulfate or cyanide. Tissue fractions exposed to 100° for 1-2 min were completely inactive. Enzymes concerned with oxidation appear to be involved in the formation of Unknown 2. Studies with eluates of I^{131}-labeled Unknown 2 from filter paper chromatograms revealed the following chemical properties: 1) Unknown 2 is not simply an oxidized form of inorganic iodide (I_2, hypoiodite, iodate). 2) It is very unstable in acid (pH 5, or less), releasing all of its I^{131} as iodide. 3) It is relatively stable in alkali. 4) It does not exchange with iodide. 5) Its I^{131} is not extractable with chloroform. Unknown 2 was not observed in vivo in rat or mouse thyroids, or in mouse salivary tissue. Only very small amounts were formed by sheep thyroid tissue slices. The physiological significance of Unknown 2, if any, remains to be determined.

1117. A MICRO METHOD FOR THE SEPARATION AND DETERMINATION OF Ca AND Sr IN BIOLOGICAL FLUIDS. Hertha H. Taussky (intr. by Roy W. Bonsnes). The Russell Sage Inst. of Pathology, Dept. of Medicine, Cornell Univ. Med. College, and New York Hosp., New York City.

The separation of Ca from Sr, ordinarily a time-consuming procedure, has been simplified into a two-step method. Ca and Sr are precipitated as the oxalates in duplicate. One precipitate is used for the determination of the sum of both Ca and Sr, the other for the separation procedure. Sr is separated from the Ca at boiling water bath temperature as the sulfate. Ca is left in solution and is then again precipitated as the oxalate. Sr is calculated as the difference between the titrations for the sum and for the Ca. The completeness of the separation of Ca and Sr is a function of the amounts of Ca and Sr present in the solution. Conditions are established under which, between certain limits of concentration, the separation is complete and coprecipitation is avoided. Direct precipitation of the oxalates from urine gives incorrect, high results because other substances also precipitate with oxalate. This interference is eliminated by preliminary treatment with iodine. Except that a new wash liquid is utilized, in which the oxalates of Ca and of Sr are practically insoluble, the final titration of the oxalates is carried out in the classical way with $KMnO_4$. However, the procedure is also applicable to EDTA titration. Recovery experiments are satisfactory.

1118. CHARACTERIZATION OF RENAL TUBULAR TRANSPORT OF GLUCOSE. W. Rowland Taylor,* Mary F. Nolan,* Claire L. Lee,* Theodore Enns* and Francis P. Chinard. Johns Hopkins Med. School and Baltimore City Hosps., Baltimore, Md.

It is generally assumed that glucose absorbed from urine of the proximal tubules is transported intact across the cells. Breakdown to lactate and resynthesis of the 6 carbon chain has been postulated. The evidence outlined below establishes that the 6 carbon chain of the transported glucose remains intact. Injection is made into the renal artery of anesthetized dogs of a solution containing T-1824, 1-C-14-glucose and creatinine. Thirty samples of renal venous blood are obtained over a period of 1 min. In 6 experiments recovery, relative to T-1824, of C-14 was 98.5±5.2%, of creatinine was 73.3±13.5%. The time-concentration curves for C-14 and creatinine differed. With prior administration of phlorhizin these differences were not obtained and the recoveries of C-14 and creatinine became nearly identical. By paper chromatography, autoradiography and enzymatic analysis it has been shown that essentially all C-14 activity is in glucose. The differences of the glucose and creatinine curves in the absence of phlorhizin accordingly should represent glucose transported by the tubule cells. Microbiological degradation of the glucose in the renal venous blood samples established that essentially all C-14 was in the glucose-1 position. The calculated transit time of glucose across the tubule cells is approximately 5 sec: the postulated carrier complex is not a macromolecule. Analysis of anaerobically collected renal venous blood samples demonstrates that less than 18% of the CO_2 produced by the kidney arises from glucose. Other data on renal CO_2 production will be presented.

1119. EFFECT OF NITROPHENOLS ON THE METABOLISM OF SPERMATOZOA. Charles Terner. Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.

The metabolism of C^{14}-labeled pyruvate, acetate and glucose by washed bull spermatozoa was studied, the respiratory $C^{14}O_2$ being used as a measure of the amount of substrate oxidized. Pyruvate was oxidized at the highest rate. Glucose oxidation was slowest with a low rate of O_2-uptake. The addition of glucose to spermatozoa metabolizing pyruvate caused depression of respiration. 2:4-Dinitrophenol (DNP, 10^{-4} M) inhibited respiration and $C^{14}O_2$ production from 2-C^{14} or 3-C^{14}-pyruvate, but accelerated respiration and $C^{14}O_2$ production from C^{14}-glucose. In concentrations of less than 3 x 10^{-4} M it did not inhibit acetate oxidation. Decarboxylation of 1-C^{14}-pyruvate was inhibited by DNP under both aerobic and anaerobic conditions. Acetate oxidation was inhibited by fluoride (0.01 M), which had little effect on pyruvate oxidation. Fluoride (0.02 M) strongly depressed respiration and $C^{14}O_2$ production from 2-C^{14}- and 3-C^{14}-pyruvate, but had no effect on the decarboxylation of 1-C^{14}-pyruvate. In contrast to DNP, p-nitrophenol did not inhibit and in concentrations from 1 x 10^{-4} - 4 x 10^{-4} M stimulated pyruvate oxidation. The high rate of pyruvate oxidation in contrast to the lower rate of glucose oxidation seems to be connected with the pyruvate dismutation reaction which is particularly vigorous in spermatozoa (Melrose and Terner. Biochem. J. 53:296, 1953). This reaction, which is sensitive to DNP, produces extra acetyl-CoA in addition to the acetyl-CoA formed by the ordinary oxidative decarboxylation of pyruvate. In the presence of glucose, the dismutation of 2 moles of pyruvate is replaced by the oxidation-reduction of triose phosphate and pyruvate without the production of extra acetyl-CoA.

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1120. ACTION OF TRANSGALACTOSYLASE OF SACCHAROMYCES FRAGILIS ON GALACTOSYL OLIGOSACCHARIDES. Carl L. Tipton,* Jean M. Marsh* and John H. Pazur. Dept. of Biochemistry and Nutrition, Univ. of Nebraska, Lincoln.

The transgalactosylase of the S. fragilis converts lactose into glucose, galactose and new galactosyl oligosaccharides of the di- and tri-saccharide types. Structural studies on the oligosaccharides isolated thus far in pure form indicate that the compounds are: 3-0-β-D-galactopyranosyl-D-glucose (I), 6-0-β-D-galactopyranosyl-D-glucose (II), 6-0-β-D-galactopyranosyl-D-galactose (III), 0-β-D-galactopyranosyl-(1-->6)-0-β-D-galactopyranosyl-(1-->4)-D-glucose (IV) and 0-β-D-galactopyranosyl-(1-->6)-0-β-D-galactopyranosyl-(1-->6)-D-glucose (V). On treatment with the enzyme, I is disproportionated to glucose, galactose, II and a trisaccharide with the probable structure, 0-β-D-galactopyranosyl-(1-->6)-0-β-D-galactopyranosyl-(1-->3)-D-glucose. II is converted by the enzyme to glucose, galactose and V. Incubation of glucose-C^{14} and V with the enzyme results in the synthesis of radioactive II most likely by a transfer of galactosyl units of V to glucose C^{14}. The transgalactosylation of II to glucose and V is evidently readily reversible. III is converted slowly to galactose and a compound with the paper chromatogram mobility of a trisaccharide. Addition of radioactive galactose to an enzymatic digest of III results in a slow incorporation of the labeled galactose into III. The reversible action of the transgalactosylase of S. fragilis draws attention to the possibility that the enzymes involved in the synthesis of many of the other biologically important galactosyl compounds (e.g. lactose, plant galactosides, galactosyl polysaccharides) may also act by reversible transgalactosylation mechanisms.

1121. ACETYL COENZYME A IN THE BIOSYNTHESIS OF FATTY ACIDS. Edward B. Titchener* and David M. Gibson. Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

Although each enzymatic step in the oxidation of fatty acids by animal tissues is reversible under appropriate conditions, the synthesis of long-chain fatty acids from acetyl CoA by the purified enzymes of the fatty acid oxidation sequence has not been demonstrated. This laboratory has previously reported a system consisting of soluble enzyme fractions from pigeon liver which actively incorporates acetate into fatty acids and which requires the addition of the following cofactors: ATP, CoA, glutathione, DPNH, isocitrate, TPN and manganese. The incorporation of C^{14}-1-acetyl CoA into fatty acids has now been demonstrated under such conditions that excess unlabeled acetate does not markedly diminish the recovery of C^{14} in the synthesized fatty acids. ATP remains, however, as a necessary cofactor. C^{14}-1-hexanoate (or hexanoyl CoA) is not appreciably incorporated into long-chain fatty acids whether or not unlabeled acetate (or acetyl CoA) is present in the system. Information will also be presented concerning the role of the various cofactors and the properties of the purified enzyme fractions in the synthesizing system.

1122. PHOSPHORYL CHOLINE IN PLANTS. N. E. Tolbert. Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

Phosphoryl choline (PC) is the major constituent in the large barium soluble-alcohol soluble phosphorus fraction from plants. Propanediol phosphate, which has previously been considered as the major constituent of this fraction, has not been detected in plants in appreciable amounts. PC reservoires, amounting to 5 to 30% of the total soluble phosphorus, show physiological changes in the roots and leaves similar to those for glutamine and asparagine. PC accumulates in roots of green or young etiolated plants, and moves in the xylem sap to the leaf where is is metabolized. As etiolated plants age, PC moves out of the roots and accumulates in large amounts in the dying leaves. There is almost complete P^{32} exchange within a few minutes to an hour after either labeled inorganic phosphate or PC is fed to green plants. Equally rapid labeling of PC with $H_2C^{14}O$ and $HC^{14}OOH$ and relatively slow labeling over 1-3 days with glycine-C^{14} or $C^{14}O_2$ photosynthesis suggest that the methyl groups also turn over at a rapid rate. Glycerol phosphoryl choline and phospholipids are labeled substantially by P^{32} only after one or more days which reflects the much slower lipid metabolism. The similarities to amide reservoires suggest that PC functions in phosphorus and methyl group storage and transport. Rapid exchange of phosphate and methyl groups suggest that PC participates in transphosphorylation and transmethylation and that these reactions may be involved in phosphate transport.

1123. EFFECT OF A-METHOPTERIN UPON PURINE BIOSYNTHESIS IN SUSCEPTIBLE AND DEPENDENT STRAINS OF L1210 LEUKEMIC TUMORS. Arthur J. Tomisek (intr. by Howard E. Skipper). Southern Research Inst., Birmingham, Ala.

For the liver, intestine, and L1210 tumors of intact mice a metabolic effect of A-methopterin inhibition has been shown to be the intracellular accumulation of 4-aminoimidazole-5-carboxamide ribotide and the exclusion of formate-C^{14} from purine derivatives. For E. coli these findings were confirmed; and in addition, some evidence was obtained showing that blockade of the de novo synthesis of purines was the "primary" site of A-methopterin inhibition. This evidence consisted of a correlation between the observed amount of intracellular accumulation of aminoimidazolecarboxamide ribotide and the degree of growth inhibition by various levels of A-methopterin. The chromatographic-autoradiographic technique employed in the above work was also found useful in revealing metabolic differences between the parent strain of L1210 leukemic cells and the A-methopterin-dependent mutant (both obtained from L. W. Law). As expected from earlier work on the nucleic acids of tumors, the effect of A-methopterin on the dependent strain of tumor was one of slightly increased formate assimilation into soluble purine derivatives. Radioactivities of the observed metabolic pool indicated that the sequence of biochemical events from formate to various purine ribotides was the same in the parent L1210 leukemia and in the A-methopterin-dependent L1210 leukemia.

1124. ISOLATION OF L-ARABITOL FROM PENTOSURIC URINE. Oscar Touster, Shirley Harwell* and Margarita Escobedo G.* Dept. of Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

L-Xylulose, the sugar excreted in essential pentosuria, has been shown to be a trace constituent of the urine of normal mammals. Furthermore, recent work in this laboratory (J. Am. Chem. Soc. 78:3544, 1956) has demonstrated the presence, in guinea pig liver mitochondria, of enzymes able to catalyze the interconversion of 1) L-xylulose and xylitol and 2) D-xylulose and xylitol. In order to determine whether xylitol is excreted in urine, deionized samples of human urine have beenchromatographed on Dowex 1

(borate) columns, the elution being followed by periodate titration as well as by colorimetric tests for sugars. Fractions containing much more periodate-positive material than accounted for by the sugar tests were obtained in the analysis of pentosuric urine. After further purification, this material was found to behave as a pentitol in all paper chromatographic tests. Acetylation yielded a crystalline product with the melting point and infrared spectrum of arabitol pentaacetate. Recrystallization of this product with an equal amount of D-arabitol pentaacetate gave the racemic compound, DL-arabitol pentaacetate, with the same melting point and infrared spectrum as an authentic sample. Although D-arabitol has been found in several natural sources, it appears that the L-isomer has not previously been encountered in nature. The origin of the L-arabitol in pentosuric urine is being investigated, as well as its possible occurrence in the urine of normal individuals.

1125. CYCLIC NATURE OF TURNOVER CURVES. S. B. Tove and H. L. Lucas (intr. by G. H. Satterfield). North Carolina Agricultural Exp. Station, Raleigh.

Previous work has shown that the turnover curves of individual liver fatty acids consists of 2 cycles having a 20-hr interval (J. Biol. Chem. 218:275, 1956). Specific activity-time curves of individual fatty acids constructed from observations taken 1, 2, 4, 6, 9 and 12 hr after injection of carboxyl-labeled acetate show 2 cycles. The first peak usually appears at 1-2 hr and the second at 6-12 hr. Similar specific activity-time curves are obtained with liver lipids, blood lipids, carcass lipids, defatted liver, defatted blood and TCA extracts of carcass. In order to reduce the high degree of variation associated with constructing a single turnover curve from points obtained from different animals, observations were made on the specific activity of expired CO_2 whereby a complete curve is obtained for each animal. Samples of expired CO_2 were collected at 15-min intervals for periods ranging to 50 hr after the injection of carboxyl-labeled acetate, 1,5-labeled citrate or bicarbonate. The results of these experiments confirm the cyclic nature of the turnover curves observed previously. The cycles which appear in the CO_2 curves are highly reproducible and appear to consist of several major cycles on which are superimposed minor cycles. The major cycles seem to occur at 20-hr intervals whereas the minor cycles seem to occur at 2.5-hr intervals. The appearance of these cycles do not seem to be correlated with the time the rats consume food or with their activity.

1126. DERIVATIVE METHOD FOR AROMATIC AMINES. Walter Troll,* Irene Leupin* and Norton Nelson. Inst. of Industrial Medicine, New York Univ., New York City.

The major metabolites of aromatic amines, as elucidated by isolation methods, appear to be amino phenols excreted mainly in the form of sulfate esters or glucuronides. Clarification of qualitative and quantitative details of the metabolic products is important, especially since the metabolic products of some amines, naphthylamine and benzidine, appear to be responsible for the formation of bladder tumors. Our approach has been to form a 1,2-naphthoquinone derivative of the amino group, which stabilizes the amino phenols and yields derivatives of the sulfate esters and glucuronides which are insoluble in dilute acid. The derivative is formed in 5 min at room temperature at pH 1. Under these conditions no other urine constituent reacts to form an insoluble precipitate, while aromatic amines react quantitatively with the reagent. Metabolites so far tested including benzidine, 3-hydroxybenzidine, the sulfate ester of benzidine, the 1-sulfate ester of naphthylamine, and benzidine glucuronide all give positive results. The precipitates are soluble in dilute alkali, can be separated by solubility in water at different pH's, by distribution into solvents, and chromatography. The benzidine-glucuronide is a substrate for glucuronidase preparation from bovine liver. Essentially all the benzidine injected intraperitoneally in a rabbit can be acounted for in the urine collected in the first 36 hr. (Aided by a grant from the Natl. Aniline Div. of the Allied Chemical and Dye Corp.)

1127. KINETICS OF AN ANTIGEN-ANTIBODY REACTION: THE LUCIFERASE-ANTILUCIFERASE SYSTEM. Frederick I. Tsuji and David L. Davis.* Research Lab., TA Hosp., and Dept. of Biophysics, Univ. of Pittsburgh, Pittsburgh, Pa.

The antigen-antibody reaction was studied using luciferase as the antigen and antiluciferase as its antibody. Luciferase was prepared as a water extract from the marine ostracod crustacean, Cypridina hilgendorfii. The source of antiluciferase was rabbit serum obtained from rabbit sensitized with luciferase. Appropriate ratios of luciferase and diluted serum were mixed in a buffered medium and aliquots were removed at various time intervals and the amount of unreacted luciferase remaining in the mixture was determined by reacting with Cypridina luciferin. In the presence of molecular oxygen, luciferin is catalytically oxidized by its enzyme luciferase, with the production of a blue luminescence whose intensity may be rapidly and accurately measured using a photomultiplier photometer. It was found that at near saturating concentrations of luciferin, the initial maximal light intensity of the luminescence was directly proportional to the amount of luciferase present in solution. The rise time to this peak intensity after mixing was in practically every case 1.2 sec or less. Consequently, this method permitted the almost instantaneous determination of free luciferase in the mixture. Furthermore, little or no dissociation of the luciferase-antiluciferase complex seemed to occur as a result of dilution during the brief interval of mixing with luciferin solution. The pH optimum of the luciferase-antiluciferase reaction was found to be 5.90 in phosphate buffer. At pH 6.80 and at room temperature (24°C), the luciferase-antiluciferase reaction appeared to obey the law of mass action and was bimolecular in nature.

1128. RETENTION OF ENZYMATIC ACTIVITY AFTER THE ACTION OF TRYPSIN OR RIBONUCLEASE. Mayo Uziel,* William H. Stein and Stanford Moore. Rockefeller Inst. for Med. Research, New York City.

In 2 M guanidine · HCl, in which ribonuclease retains its activity as it does in 8 M urea (Anfinsen. Compt.-rend. Lab. Carlsberg, Ser. Chim. 30:13, 1956), tryptic hydrolysis at pH 6 and 30° leads to a 40 o/o increase in the ninhydrin color value and the retention of more than half of the enzymatic activity. The hydrolysate was dialyzed against pH 5 buffer at 4° to remove the guanidine and chromatographed at pH 6.1 on IRC-50 at 4°. The activity (as measured against cyclic cytidylic and uridylic phosphates) was present in a peak that emerged after the position of ribonuclease A. The new product possessed the same amino acid composition and molar extinction at 278 and 284 mμ as ribonuclease; the ninhydrin color value however, was 30-40 o/o greater than that of ribonuclease based on the same total quantity of amino

acid residues. Trypsin apparently can cleave 3-5 peptide bonds in the ribonuclease molecule without releasing any amino acid residues and without causing a major reduction in the enzymatic activity. From the partial structural formula (Hirs et al. J. Biol. Chem. 221:151, 1956) it can be concluded that hydrolysis without loss of amino acids could occur at a maximum of 5 lysyl and arginyl bonds provided that only 1 bond between any 2 half-cystine residues is split. Further work will determine whether tryptic hydrolysis can lead to more extensively degraded products still possessing ribonuclease activity (cf. Richards on hydrolysis by subtilisin, Compt.-rend. Lab. Carlsberg Ser. Chim. 29:315, 1955, and Anfinsen on cleavage by pepsin, J. Biol. Chem. 221:405, 1956).

1129. SPECTROPHOTOMETRIC EVIDENCE FOR ENZYMEZINC-INHIBITOR COMPLEXATION. Bert L. Vallee, Thomas L. Coombs* and Robert J. P. Williams.* Biophysics Research Lab., Dept. of Medicine, Harvard Med. School, Boston, Mass.

The detection of zinc in several enzymes has led to the proposition that this metal is functional in the reactions catalyzed by these enzymes (Adv. Prot. Chem. 10:317, 1955). This has been inferred from data indicating the simultaneous aggregation of zinc and of enzymatic activity during isolation of these enzymes, accompanied by the disappearance of other metals. Chelating agents known to form complexes with ionic zinc in aqueous systems have been employed to inhibit enzymatic activity, and the kinetics of this inhibition have been examined, particularly with 1,10-phenanthroline. Such studies further imply an essential role for zinc. 1,10-Phenanthroline forms mono, bis, and termolecular complexes with zinc, the characteristics of which have been described (J. Am. Chem. Soc. 73:391, 1951). We have found higherto unrecognized absorption maxima at 3275 and 3425 A for these systems. The extinction coefficients of the 3 molecular species at these 2 wavelengths have been calculated. The addition of 1,10-phenanthroline to various zinc-containing enzymes and zinc-insulin has demonstrated changes in absorption at 3275 and 3425 A. The direct interaction of 1,10-phenanthroline with the protein-bound zinc has thus been demonstrated, simultaneously permitting the quantitative determination of the metal. The stoichiometric relationships of zinc and 1,10-phenanthroline have been studied in these enzymes and correlated with the inhibition data. Spectrophotometric studies of zinc enzymes with other chelating agents also demonstrate changes characteristic of their ionic zinc systems.

1130. EFFECTS OF REFEEDING UPON IN VIVO AND IN VITRO LIPOGENESIS. John T. Van Bruggen and Robert J. Emerson.* Biochemistry Dept., Univ. of Oregon Med. School, Portland.

Young adult, trained-fed rats were refed a standard chow meal after a 13-hr fast. Intact rats and liver slice preparations were studied at fixed periods following the chow meal using acetate-1-C^{14}. Information obtained on the lipogenic activity of liver, gut, carcass and skin extends data previously presented (Fed. Proc. 14:295, 1955) and demonstrates the lability of lipogenic reactions. Use of animals for lipogenic studies without knowledge of their nutritional status just prior to use is shown to be hazardous. The response of tissues in vivo is compared with liver slice preparations. The lipogenic response of hepatic tissue of the 2 preparations is of a similar magnitude and the response to fasting is also comparable. Changes of the order of 10-fold in fatty acid labeling occur after feeding. $C^{14}O_2$ information obtainable from the slice preparation indicates little effect of feeding upon tracer utilization in reactions producing CO_2. In both preparations, the changes seen in lipid labeling are greater in the fatty acid fraction, compared to cholesterol fractions. (Done under contract with the Atomic Energy Comm.)

1131. MECHANISM OF PYRIDINE NUCLEOTIDE DEPENDENT DEHYDROGENASES. Jan Van Eys (intr. by Nathan O. Kaplan). McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

It has been found that the Michaelis-Menten constants for substrate range from zero to infinity for enzymes which catalyze the transfer of hydrogen from alcohols or primary amines to DPN (for example alcohol-, lactic-, or glutamic dehydrogenases). The K_m is proportional to the reciprocal of the coenzyme concentration. The K_m for the coenzyme is equally dependent on the concentration of substrate. Similarly, different constants can be obtained at a given level of coenzyme, when acetylpyridine-DPN or pyridinealdehyde-DPN are used as coenzymes in place of DPN. Nucleophilic compounds, structurally related to the substrate, form ternary complexes with coenzyme and enzyme (van Eys et al. Biochim. Biophys. Acta, in press), and when it can be determined, the stoichiometry shows maximally 1 mole of substrate per 2 moles of DPN. These dehydrogenases show either substrate inhibition or activation and, furthermore, in some cases it can be shown that more than 1 DPN is required per mole of enzyme before the reaction proceeds. To explain these data, the following reaction sequence is proposed: the enzyme-DPN complex (containing more than 1 DPN) binds the substrate to the para-position of 1 DPN molecule, through the hydroxyl or aminogroup, with the release of a hydrogen ion. A second molecule of DPN acts, by direct transfer, as the recipient of the hydrogen from the substrate. When the product is released, the first DPN is regenerated, while the second DPN is converted to DPNH. All DPN molecules on the protein are equally capable of acting as hydrogen or substrate acceptor.

1132. RELATIONSHIP OF DIETARY PROTEIN, ASCORBIC ACID AND GLUTATHIONE IN ESTROGEN INACTIVATION. Frank D. Vasington and Raymond E. Vanderlinde (intr. by Emil G. Schmidt). Dept. of Biochemistry, Univ. of Maryland School of Medicine, Baltimore, Md.

An adequate intake of dietary protein has been reported to be necessary for estrogen inactivation both in vivo (Jailer and Seaman. Proc. Soc. Exp. Biol. & Med. 73:70, 1950; Vanderlinde and Westerfeld, Endocrinology 47:265, 1950) and in vitro (Unna et al. Proc. Soc. Exper. Biol. & Med. 55:254, 1944). Liver glutathione and ascorbic acid rapidly decrease in rats on a protein-free diet (Klug et al. Proc. S. Dakota Acad. Sci. 29:38, 1950; Edwards and Westerfeld, Proc. Soc. Exp. Biol. & Med. 79:57, 1952) and a low protein diet has been reported to cause a decreased synthesis of ascorbic acid in the white rat (Yanovskayo. C.A. 47:718h, 1952). It therefore appeared that ascorbic acid and glutathione might be involved in estrogen metabolism. We found the daily injection of glutathione to have no effect on the inability of rats on a low protein diet to inactivate implanted estrone pellets as measured by vaginal smear response, but the daily injection of 50 mg of ascorbic acid resulted in estrogen inactivation. However, liver slices from rats maintained on the same low protein diet for approximately 3 months were able to inactivate estradiol. In addition, ascorbic, dehydroascorbic, isoascorbic and glucoascorbic acids at the 10 mg level were found to actually

inhibit estradiol inactivation by liver slices, whereas glutathione had no effect. These in vitro observations are in direct contrast to our in vivo observations and the in vitro results of Unna et al. (1944). Scorbutic guinea pig liver slices were also able to inactivate estradiol under the same conditions. As with rat liver slices, ascorbic acid at the 10 mg level inhibited estrogen inactivation.

1133. PHOTOCHEMICAL REDUCTION OF LOW POTENTIAL COMPOUNDS BY LEAF PREPARATIONS. Leo P. Vernon and M. Odell Hobbs.* Dept. of Chemistry and Chem. Engineering Science, Brigham Young Univ., Provo, Utah.

In the presence of ascorbate and 2,6-dichlorophenolindophenol (DPIP) illuminated leaf homogenates reduced indigo carmine (E'_o = -.125 at pH 7) and flavin mononucleotide (E'_o = -.185 at pH 7). The photoreduction of indigo carmine proceeded rapidly in the presence of air while anaerobic conditions were necessary for flavin mononucleotide photoreduction. Both reactions were strictly dependent upon the presence of ascorbate and DPIP, indicating that ascorbate maintained DPIP in the reduced form which reacted with the photochemical OH system, leaving the photochemical H system free for the reductive process. Balance studies revealed the ratio of ascorbate oxidized to indigo carmine reduced to be close to 1. Chloroplasts isolated by centrifugation had the ability to catalyze the reactions, but to a lesser degree. Addition of supernatant fluid increased chloroplast activity to the level observed for leaf homogenates. Reduction of DPN by illuminated chloroplasts took place at a much slower rate, required high concentrations of chloroplasts and DPN, and was not dependent upon the presence of ascorbate and DPIP.

1134. PROGESTERONE AS A PRECURSOR OF HUMAN TESTICULAR ANDROGENS. Thomas A. Viscelli, Michael E. Lombardo and Perry B. Hudson (intr. by W. M. Sperry). Inst. of Cancer Research, Depts. of Biochemistry and Urology, College of Physicians and Surgeons, Columbia Univ., New York City.

Recent investigations by Lynn (Fed. Proc. 15:305, 1956) and Slaunwhite and Samuels (J. Biol. Chem. 220: 457, 1953) using rat and bovine testicular tissue indicate the possible role of progesterone in androgen biosynthesis. Radioactive progesterone was the substrate in each case. In our laboratories, human testicular tissue was obtained at surgery, and the capsules removed. The glands were minced in cold Krebs-Ringer phosphate buffer pH 7.4 with the following additions: 0.68 mM fumarate; 0.02 mM ATP and 18 mg DPN. Progesterone was added in a ratio of 1 part to 2000 parts of tissue in approximately 0.5 ml ethyl alcohol. The suspensions were incubated for 3 hr at 38°C under an atmosphere of 95% O_2/5% CO_2. Controls were prepared in the same way, without added substrate. The incubates were extracted by dialysis coupled with continuous extraction, dried and chromatographed in hexane saturated with propylene glycol for periods of from 72-8 hr. The Mineralite lamp and the Zimmermann reagent were employed to detect steroid-containing zones on the papergrams. Ultraviolet-absorbing areas corresponding to 17α-hydroxyprogesterone were detected in each instance, and another corresponding to testosterone in one instance. Another area more polar than 17α-hydroxyprogesterone was detected. Preliminary studies indicate that this substance may correspond to the C_{17}-C_{20} glycol reported by Lynn. The amounts were calculated on the basis of their optical densities at 239-242 mμ, using the extinction coefficient of the authentic steroid in the same solvent. The identification was based on chromatographic behavior, including mixed chromatograms with standard compounds, ultraviolet absorbing properties and sulfuric acid chromogens absorbing from 220-600 mμ. Conversion of progesterone to 17α-hydroxyprogesterone was of the order of 3.7%, and 0.7% for testosterone. The "glycol" represents a conversion of approximately 1.0%.

1135. RIBOSE FORMATION FROM ACETATE IN ALKALIGENES FAECALIS. Wolf Vishniac and Faith N. Brenneman.* Dept. of Microbiology, Yale Univ., New Haven, Conn.

The report of Pinchot (J. Biol. Chem. 205:65, 1953) that cell-free extracts of Alkaligenes faecalis did not metabolize hexose phosphates prompted a study of the carbohydrate metabolism of this organism. Vigorous growth occurs on acetate as the only carbon source, and the mode of acetate incorporation into the ribose of RNA was followed in whole organisms and in alumina-ground extracts of A. faecalis. Preparations were incubated with acetate-2-C^{14}, extracted with perchloric acid, and hydrolyzed with KOH. Ribose was isolated from the hydrolyzate by ion-exchange chromatography in borate buffer and was found to be radioactive. After treatment with Dowex-1 the ribose was degraded by fermentation with Lactobacillus plantarum, in which carbons 1 and 2 give rise to acetate and carbons 3, 4, and 5 lactate. Of the total radioactivity in the ribose one third occurred in carbons 1 and 2, and two thirds in carbons 3, 4, and 5. Upon incubation of cell-free extracts simultaneously with acetate-2-C^{14} and unlabeled glyoxalate 95% of the radioactivity in ribose was found in carbons 3, 4, and 5, suggesting that in this reaction mixture glyoxalate displaces acetate in the formation of carbons 1 and 2 of ribose. In addition, acetate and glyoxalate give rise to significant amounts of malate, presumably by a reaction similar to that reported by Ajl and Wong (J. Am. Chem. Soc. 78:3230, 1956), and pyruvate. Possible routes of ribose synthesis in A. faecalis will be discussed. (Supported by the Natl. Science Fndn. and the Natl. Insts. of Health, PHS.)

1136. OXIDATION STATE OF RESPIRATORY CARRIERS AND THE ATP-P_i^{32} EXCHANGE REACTION. Charles L. Wadkins* and Albert L. Lehninger. Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Md.

To account for coupling of phosphorylation to electron transport from substrate to oxygen it has been proposed that at 3 points in the respiratory chain oxido-reduction steps lead to the formation of a "high-energy" linkage between a respiratory carrier and phosphate or with some other group with which phosphate may exchange. Such a "high-energy" complex of the respiratory carrier may be regarded as the ultimate donor of phosphate to ADP. Thermodynamic considerations alone do not suffice to determine whether such a primary "high-energy" complex involves the oxidized or the reduced state of the carrier. Experiments designed to determine which oxidation state is involved have been performed with a complex of electron transport and phosphorylating enzymes separated from digitonin extracts of liver mitochondria. Such preparations catalyze an exchange of P_i^{32} with the terminal phosphate of ATP in the absence of net electron transfer. This exchange has been found to be dependent on oxidation-reduction state under certain experimental conditions. The exchange is maximal when the carriers are in the oxidized state and is nearly completely inhibited when the carriers

are in the reduced state. The oxidized state of the carriers thus appears to be the form which ultimately donates the "high-energy" linkage in the coupling of phosphorylation to electron transport.

1137. ISOLATION OF PHOSPHATIDYL INOSITOL FROM PEAS. A. C. Wagenknecht* and H. E. Carter. Div. of Biochemistry, Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.

In the course of a study of the lipides of green peas (Pisum sativum, var. Thomas Laxton) lyophilized raw peas were ground to pass 40 mesh and extracted with chloroform-methanol 3:1 at room temperature. The crude pea lipides (6.0% on dry weight basis) were fractionated with acetone into acetone soluble (47%) and acetone insoluble (53%) fractions. The acetone insoluble fraction was treated twice with 10 volumes of glacial acetic acid; the material insoluble in acetic acid was collected by centrifuging and suspended in water. The aqueous suspension was extracted twice with benzene, and the mixture was centrifuged to remove insoluble material. The benzene solution was lyophilized to a white powder (11.4% of acetone insoluble, 5% of total pea lipides). The main constituent of this material appeared to be the mixed calcium-magnesium salt of phosphatidyl inositol. The products of acid and milk alkaline hydrolysis were shown by paper chromatography to include inositol monophosphate, glycerophosphate, inorganic phosphate, inositol and glycerol. Analyses of carbon, hydrogen, phosphorus, glycerol and ash, and determinations of saponification equivalent, fatty acid content and iodine number all agree with this postulation. The fatty acids (mean molecular weight of 260; iodine value of 49.6) correspond to a mixture of myristic and oleic acids. These data indicate that pea lipides constitute a convenient lipide source from which relatively pure phosphatidyl inositol can be isolated by a simple solvent fractionation procedure.

1138. DERIVATION OF EMBRYONIC CHICK PROTEINS. Harry Walter* and H. R. Mahler. Dept. of Chemistry, Indiana Univ., Bloomington.

We have injected chicken proteins (serum globulin, serum albumin and ovalbumin) their peptides, and amino acids, all radioactively labeled with S^{35} cystine and methionine, into the egg whites or yolks of unincubated, embryonated eggs. After 5-9 days' incubation the embryos were harvested and the radioactivity in their proteins was determined (cf. Nature 178:1176, 1956). The extent and rate of incorporation of injected activity per embryo varies as follows: amino acids $\cong$ peptides >> proteins when these embryonic protein precursors are injected into the egg white; and amino acids $\cong$ peptides $\cong$ proteins when the injection is into the yolk. Furthermore amino acids and peptides are used equally well whether injected into the egg white or into the yolk. In the absence of permeability or other pool barriers, labeled, ibjected protein can be considered a tracer for the egg white protein or yolk protein in the egg (depending on the mode of injection). Thus, when the weight of total yolk proteins is multiplied by the percentage of injected activity found per embryo, a value for the expected protein per embryo is obtained. This calculated value is, however, 2-3 times larger than the amount of embryonic protein as determined by actual analysis. Similar calculations based on total egg white proteins lead to only a very small fraction (0.02-0.15) of the embryonic protein content actually observed. The data may be interpreted that over the time period studied the egg white proteins are not embruonic protein precursors; that soluble proteins are utilized for this purpose from the yolk and preferentially to some of the yolk proteins.

1139. CARBOHYDRATE METABOLISM IN PENICILLIUM DIGITATUM. C. H. Wang, D. J. Reed,* E. Noble,* B. E. Christensen* and V. H. Cheldelin. Oregon State College, Corvallis.

The mechanism of glucose catabolism in P. digitatum was studied by the use of C^{14} labeled glucose, pyruvate, acetate and CO_2. In the presence of glucose, $C^{14}O_2$ was found to be incorporated into C-1,-2 and -4 of aspartic acid, and C-1 and C-(2,3,4) of glutamic acid. Carboxyl labeled acetate was utilized slowly by this organism resulting in the labeling of C-1,-2 and -4 of aspartic acid, and C-1, -(2,3,4) and -5 of glutamic acid. C-2 and C-6 of glucose gave rise to the amino and methyl carbons of alanine which points to pyruvate as the precursor, produced via glycolysis. C-2 of glucose was incorporated into the C-3 of aspartic acid. The results suggest that CO_2 fixation resembling a $CO_2+C_2+CO_2$ combination is operating in this organism. The C_2 unit could have been derived from C-2 and -3 of glucose by way of pentose formation and a subsequent C_3-C_3 cleavage. Time course studies with respect to radiochemical recovery in CO_2 and cells from the labeled substrates gave the following picture: in respiratory CO_2, 46, 35, 48 and 30% from glucose-1,-2,-3(4) and -6-C^{14} respectively; 59, 50 and 35% from pyruvate-1,-2 and -3-C^{14}; and 48 and 35% from acetate-1 and -2-C^{14}. Incorporation into the cells was 44, 56, 41 and 53% from glucose-1, -2, -3(4) and -6-C^{14}, respectively. These data suggest the operation of glycolysis, a "direct" oxidative pathway and a limited Krebs cycle in this organism. The distribution of the catabolic pathways of glucose in P. digitatum was estimated according to the method of Wang et al. (J. Am. Chem. Soc. 78:1869, 1956) to be 80-82% glycolysis and 18-20% "direct" oxidative pathway.

1140. CHROMATOGRAPHY OF LUTEINIZING HORMONE (LH) ON CELLULOSE ION EXCHANGERS. Darrell N. Ward and Elizabeth E. Neu (intr. by A. Clark Griffin). Dept. of Biochemistry, Univ. of Texas, M. D. Anderson Hosp. and Tumor Inst., Houston.

Leuteinizing hormone from sheep pituitary glands has been prepared by methods reported in the literature. These partially purified preparations have been subjected to chromatography on both DEAE- and CM-cellulose ion exchangers prepared according to the method of Peterson and Sober. Biological activity of the LH has been evaluated qualitatively by the frog sperm-release test and quantitatively using the rat ventral prostate growth in hypophysectomized animals. The best preparation obtained from the chromatography is approximately 130% of the potency of Armour sheep LH (Lot 227-80). Chromatography of the Armour material revealed an impurity amounting to 25% of the starting weight. Data characterizing the above LH preparation with respect to electrophoresis and ultracentrifugation will be presented.

1141. INTERACTION OF POLYADENYLIC AND POLYURIDYLIC ACIDS. Robert C. Warner. Dept. of Biochemistry, New York Univ. College of Medicine, New York City.

The interaction of polyadenylic and polyuridylic acids with the formation of a stable aggregate of high molecular weight has been previously described (Fed. Proc. 15:379, 1956). Some additional properties of this aggregate and of the reaction by which it is formed have been investigated. On raising the temperature of a solution of the aggregate in phosphate buffer at pH 7 a gradual increase in optical density occurs at 258 mμ, followed by a sharp increase at 58°. The effect is reversible and on cooling the original optical

density is recovered. Similar changes occur when solutions of polyadenylic, polycytidylic or polyinosinic acids are heated alone. In these cases higher temperatures are required to produce the changes and they do not occur at such a sharply defined temperature. All of the temperature relations vary somewhat with the molecular weight of the polymer. Experiments have also been carried out on the effect of urea and ionic sternth on the aggregation reaction both at room and at elevated temperatures. Urea produces a partial dissociation of the aggregate and a lowering of the temperature at which dissociation is complete as indicated by the changes in optical density. These are also reversible and a lowering in extinction is obtained on reducing the urea concentration by dilution or dialysis. The increase in extinction in urea and on raising the temperature may be interpreted in terms of the breaking of hydrogen bonds between the purines and pyrimidines.

1142. TRANSFORMYLATION AND PURINE BIOSYNTHESIS. Leonard Warren (intr. by D. F. Waugh). Div. of Biochemistry, Massachusetts Inst. of Technology, Cambridge.

Two purified transformylases from chicken liver are responsible for the transfer of the formyl group of anhydroleucovorin to an acceptor, yielding tetrahydrofolic acid (THFA). GAR transformylase converts irreversibly, glycinamide ribotide (GAR) to formylglycinamide ribotide (FGAR), Reaction (1): Anhydroleucovorin + GAR⟶FGAR + THFA. The AICAR transformylase preparation converts 5-amino-4-imidazolecarboxamide ribotide (AICAR) to 5-formamido-4-imidazolecarboxamide ribotide (FAICAR) and then closes the ring enzymatically to form inosinic acid (IMP). Reaction (2): IMP + THFA⇌ICAR + Formyl THFA. Reaction (2) is reversible and requires K^+ ions. FAICAR can substitute for IMP in reaction (2). Together, both purified enzymes catalyze reaction (3): IMP + GAR⟶AICAR + FGAR. Homocysteine or ascorbate, K^+ ions and catalytic amounts of reduced folic acid compound are required for this combined reaction. Reaction (1) proceeds to completion. For the GAR that disappears equivalent amounts of FGAR and THFA are formed. THFA breaks down non-enzymatically to a pteridine, formaldehyde and p-aminobenzoyl glutamate which is measured by the Bratton-Marshall reaction. Racemic anhydroleucovorin, N_{10} formyltetrahydrofolic acid and acid treated leucovorin transformylate completely in the presence of excess amounts of GAR. Synthetic leucovorin and natural citrovorum factor, enzymatically activated with ATP, are utilized to the extent of 50 and 100%, respectively. Since all of the synthetic compounds exist as racemates due to stereoasymmetry of carbon 6 of the pteridine ring, a satisfactory explanation must be found for enzymatic utilization of both forms in the enzymatic reaction.

1143. PLACENTAL TRANSFER OF CALCIUM AND STRONTIUM IN THE RAT AND RABBIT. R. H. Wasserman,* C. L. Comar, M. M. Nold* and F. W. Lengemann.* Med. Div., Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.

The comparative metabolism of calcium and strontium during fetal development was investigated in rats and rabbits using double tracer experiments with Ca 45 and either Sr 85 or Sr 89. Upon feeding the isotopes to pregnant rats for about 5 days until birth, it was found that the relative Sr^*/Ca^* ratios in the fetus and maternal skeleton were 0.17 and 0.28 respectively, when the dietary Sr^*/Ca^* ratio equalled 1. The over-all discrimination of 0.17 between fetus and diet resulted from an absorptive Sr^*/Ca^* discrimination factor of 0.42, a urinary Sr^*/Ca^* discrimination factor of 0.63 and a fetal Sr^*/Ca^* discrimination factor of 0.65. The site of fetal discrimination was observed to be the placental barrier; the major discrimination occurred in the movement of Ca^* and Sr^* from dam to fetus with little or no differential metabolism from fetus to dam. The rabbit placenta was about twice as permeable to Ca^* than Sr^*, being similar in this respect to the rat. In the rat experiments, it was estimated that 92% of the fetal calcium had originated from the maternal diet. It was also determined that, in the rabbit in late pregnancy, about 24 mg calcium/fetus/day moved across the placenta as compared with an estimated calcium accretion rate of about 13 mg/fetus/day.

1144. STEADY STATE CHANGES OF THE CYTOCHROMES FOLLOWING ISOMETRIC TWITCHES. Annemarie Weber (intr. by David Nachmansohn). Dept. of Neurology, Columbia Univ., New York City, and Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.

In continuation of Connelly's and Chance's studies on frog sartorius the steady state of the cytochromes in rest and during activity was investigated. Reduction of the cytochromes by anaerobiosis led to an estimate of the concentrations of the cytochromes a_3, a, b, and c of 2 to 3 x 10^{-6} mol/kg. Under aerobic conditions antimycin-A caused a reduction, amytal an oxidation of cytochrome b. Thus b was estimated to be reduced to about 30-40% in the resting muscle. Even in glucose-bicarbonate Ringer b became more reduced after the addition of pyruvate or lactate as substrates. It became more oxidized after treatment with iodoacetic acid plus extensive stimulation which presumably lead to an exhaustion of endogenous substrate. In the presence of pyruvate isometric twitches produce consistently an oxidation of cytochrome b analogous to the oxidation found by Chance when ADP was added to mitochondria previously saturated with substrate. The return to the resting steady state took about 2-20 min depending on the temperature and the number of twitches for half the maximal effect was between 5 and 7. In the presence of azide, stimulation produced a reversible reduction of a_3 (Chance and Williams). Thus 4 components of the respiratory chain were found to shift their steady state oxidation reduction levels in response to a muscle twitch in the same way as isolated rat heart sarcosomes are found to respond to the addition of ADP.

1145. AMINO ACID INCORPORATION INTO THE PROTEINS OF DISRUPTED RIBONUCLEOPROTEIN PARTICLES. George C. Webster. Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

Microsomal ribonucleoprotein particles from pea seedlings (Tso et al. J. Biophys. Biochem. Cytology, 1956) have been found to catalyze: 1) the incorporation of amino acids into their protein; 2) the incorporation of nucleoside-5'-phosphates into their ribonucleic acid; 3) the activation of at least 12 amino acids; and 4) under certain conditions, an amino acid-dependent exchange of AMP with ATP. Unlike liver microsomes, the particles do not require either guanosine phosphates or additional protein fractions for amino acid incorporation. The particles are disrupted by treatment with concentrated salt solutions, ATP, pyrophosphate or sonic vibration. Of these treatments, only sonic vibration produces preparations that catalyze amino acid incorporation into protein. The disrupted-particle preparations can be separated into 3 groups

of fractions by ammonium sulfate treatment. These are: 1) a group of nucleoproteins of varying size, some of which catalyze all 4 activities listed above, while others catalyze only the amino acid activation and AMP-ATP exchange reactions; 2) a group of inactive proteins; and 3) an inactive fraction containing almost 50% of the RNA of the original particles in the form of small, non-precipitable nucleotides attached to a small amount of protein. The components of the active nucleoprotein fraction are being separated and employed in studies on the mechanism of protein synthesis.

1146. PROTEOLYTOC ENZYME SYSTEM OF PANCREAS. Leopold Weil and Marie Telka.* Eastern Regional Research Lab., Philadelphia, Pa.

Pancreas protaminase, described years ago (Ztschr. f. physiol. Chem. 197:219, 1931; J. Biol. Chem. 105: 291, 1934) is not identical to any known proteolytic enzymes of pancreas. A method for the preparation of protaminase from fresh pancreas by selective adsorption is given. The action of protaminase is confined to the liberation of free arginine from the C-terminal end of protamines. The action of this enzyme is not limited to the intact salmine molecule, but also extends to its tryptic and chymotryptic breakdown products. In both cases its action is accompanied by liberation of free arginine. Protaminase undergoes marked activation upon addition of traces of trypsin. This additional increase in activity is shown not to be due to protaminase but to a new enzyme. Heat treatment of the activated protaminase at pH 2.3 at 95° results only in a partial inactivation, leaving behind a "heat-stable" enzyme which resembles protaminase in that it hydrolyzes salmine or clupein but does not liberate free arginine. Similar heat treatment of protaminase itself destroys the activity of this enzyme. Five protamines tested so far have been found highly susceptible to both enzyme action. Preliminary specificity studies are presented.

1147. STABILITY OF AEROBIC PHOSPHORYLATION IN MITOCHONDRIA. Eugene C. Weinbach. Natl. Inst. of Allergy and Infectious Diseases, Bethesda, Md.

Although it has been observed that isolated liver mitochondria, after prolonged storage at low temperatures, lose their capacity for oxidative phosphorylation, we have found that mitochondria from livers of Sprague-Dawley rats retain their ability to couple phosphorylation to the oxidation of β-hydroxybutyrate after storage for periods up to 72 hr at 5°. The mitochondria were isolated in 0.25 M sucrose essentially by the procedure of Schneider et al. No chelating agents or inert proteins were required to stabilize the mitochondria. Almost full activity was retained after 24 hr storage; however, there was a gradual decline in the phosphorylating activity during the remainder of the 72-hr period, when approximately 40% of the original activity remained. The decline in the coupled phosphorylation was paralleled by increased ATP dephosphorylating ability of the mitochondria and a loss of latency. Neither the freshly isolated mitochondria nor those aged at 5° for 24 hr required added DPN. Furthermore, there was no change in the turbidity measurements of the mitochondrial suspension held at low temperatures for 24 hr, indicating that little or no swelling of the mitochondria had occurred during aging. However, the marked dependence of substrate oxidation upon added inorganic phosphate and phosphate acceptor (ADP) observed with the fresh preparations was diminished with the aged mitochondria. The experiments imply that these mitochondria, when freshly prepared, are "tightly coupled" and only slowly become "uncoupled" during prolonged storage at low temperatures. Preliminary experiments with mouse liver mitochondria indicate less stability than those of rat liver.

1148. METABOLISM OF α-AMINO-β-KETOADIPIC ACID. Irving Weliky* and David Shemin. Dept. of Biochemistry, Columbia Univ., New York City.

We have previously demonstrated that δ-aminolevulinic acid is the source of the carbon and nitrogen atoms of protoporphyrin and that the δ-carbon atom of this aminoketone has the same metabolic spectrum as that known for the α-carbon atom of glycine. Furthermore, we have postulated that α-amino-β-ketoadipic acid is an obligatory intermediate in the formation of δ-aminolevulinic acid from succinate and glycine. This communication deals with the metabolism of α-amino-β-ketoadipic acid. The diethyl ester of DL α-amino-β-ketoadipic acid-2-C^{14} have been synthesized and their metabolic patterns studied (Shemin., Ciba Fndn. Symp.; Porphyrin Biosynthesis and Metabolism; London: Churchill, 1955; 3rd Int. Congress of Biochemistry, Brussels; p. 197, 1955; Neuberger, Scott and Shuster, Biochem. J. 64:137, 1956). α-Amino-β-ketoadipic acid can be split to yield glycine as evidenced by the formation of radioactive hippuric acid after the administration of the diethyl ester of DL α-amino-β-ketoadipic acid-2-C^{14} along with benzoic acid to rats. The compound also gives rise to the excretion of porphobilinogen after injection into a rat. It was further found that the amino keto acid is almost as well utilized for porphyrin formation, as in δ-aminolevulinic acid, in duck erythrocyte preparations, if one includes, a water extract of an acetone powder prepared from rat liver, in the incubation mixture. The rat liver preparation contains an esterase not found in the duck erythrocytes. The liver preparation yields 0.5 moles of radioactive CO_2 for each mole of the diethyl ester of D2-α-amino-β-ketoadipic acid-1-C^{14}. Presumably only the L-form is deesterified and the free β-keto acid is then decarboxylated. Uric acid excreted by a pigeon injected with the diethyl ester of α-amino-β-ketoadipic acid-2-C^{14} is found to be radioactive; 89% of the total radioactivity of the uric acid resided in carbon atoms 2 and 8 whereas carbon atom 5 contained 10% of the total radioactivity found in the uric acid. The aminoketo acid is readily metabolized by rat liver slices. The diethyl ester of α-amino-β-ketoadipic acid-2-C^{14} yields radioactive CO_2 whose activity is far greater than that from δ-aminolevulinic acid and about equal to that formed from glycine-2-C^{14}. Whereas as unlabeled glycine or glyoxylate does not decrease the radioactivity of the CO_2 formed from the α-carbon atom of the aminoketo acid, the radioactivity of the CO_2 formed from glycine-2-c^{14} in the presence of the unlabeled diethyl ester is markedly lowered.

1149. SODIUM DIETHYLDITHIOCARBAMATE IN THE TREATMENT OF NICKEL POISONING. Bob West* and F. William Sunderman. Div. of Metabolic Research, Jefferson Med. College, Philadelphia, Pa.

The therapeutic effectiveness of sodium diethyldithiocarbamate has been studied in experimental animals receiving lethal amounts of nickel carbonyl and inorganic nickel salts. In previous studies from our laboratory the LD_{50} values for mice, rats and cats after 30-min exposures to nickel carbonyl were found to be 0.067, 0.24 and 1.9 mg/l. respectively. The present studies were made on mice, rats and rabbits. Mice were exposed to nickel carbonyl in concentrations of 0.06 to 0.09 mg/l. One half of the exposed mice were untreated and the other half received sodium

diethyldithiocarbamate in doses from 50-100 mg/kg of body weight immediately after exposure. No fatalities occurred in mice treated with sodium diethyldithiocarbamate, whereas 80% of the untreated mice died. In rats and rabbits exposed for 30 min to concentrations of 0.25 and 1.5 mg/l. of nickel carbonyl, respectively, similar protection was provided exposed animals receiving sodium diethyldithiocarbamate parenterally in doses of 100 mg/kg of body weight. This compound was also found to protect mice receiving lethal amounts of nickel nitrate. Preliminary studies indicate that sodium diethyldithiocarbamate is therapeutically effective when administered orally. It has been found that larger amounts of nickel are excreted in the urine and feces of rats exposed to the vapors of nickel carbonyl and treated with sodium diethyldithiocarbamate than in surviving untreated animals similarly exposed. Sodium diethyldithiocarbamate, per se, is relatively non-toxic, - the LD_{50} value by either oral or i.p. administration being approximately 1.5 gm/kg of body weight.

1150. FEEDING EXPERIMENTS WITH MERCAPTURIC ACIDS. Harold D. West, Richard A. Ewing, Jr.,* Charles Mosee,* John F. Williams* and Isaac H. Miller.* Dept. of Biochemistry, Meharry Med. College, Nashville, Tenn.

From urines of Wistar strain albino rats administered three isomeric monochlorophenyl-, three isomeric monobromophenyl- and α-and β-naphthyl-L-cysteines at a level of 2% in a synthetic diet could be isolated corresponding mercapturic acids and oils convertible to corresponding crystalling p-toluenesulfonyl-phenol derivatives. α-Naphthyl-, p-chlorophenyl- and p-bromophenylmercapturic acids fed under similar conditions could be recovered in part from the urines and, in addition, oils giving the corresponding crystalline p-toluenesulfonyl-phenol derivatives. p-Chlorophenyl-DL-homocystein and p-chlorophenyl-DL-homomercapturic acid were synthesized and administered at levels of 0.5%. In each case oils were obtained which yielded crystalline p-chlorophenyl-4-toluenesulfonate. Exhaustive chloroform extraction produced no mercapturic acid. If p-chlorophenyl-DL-homocysteine and p-chlorophenyl-DL-homomercapturic acid were incorporated at a level of 2%, however, both p-chlorophenyl-DL-homomercapturic acid and an oil which gave the crystalline p-toluenesulfonyl-derivative of p-chlorophenol could be isolated. Diphenylmercapturic acid added to diets at a level of 1% was not excreted in the urine. Crystalline 4-hydroxydiphenyl and p-β-D-glucuronosidodiphenyl, however, could be isolated. S^{35}-DL-methionine was administered parenterally to animals ingesting p-chlorophenylmercapturic acid and p-bromophenylmercapturic acid and chloroform extraction resulted in the isolation of S^{35}-p-chlorophenylmercapturic acid and S^{35}-p-bromophenylmercapturic acid. Experiments with S^{35}-DL-methionine and S^{35}-L-cysteine fed to rats receiving diphenyl indicated that increasing sulfur-containing amino acid supplements does not increase the amount of chloroform extractable sulfur-35. Compounds were identified by determination of melting points, mixed melting points, microanalyses or comparison of their infrared spectrograms with authentic samples. (Supported in part by Research Grant C-1524 from the Natl. Cancer Inst., PHS.)

1151. SPECTROPHOTOMETRIC OBSERVATIONS ON INTERACTION BETWEEN PROTEINS AND Δ^4-3-KETOSTEROIDS. Ulrich Westphal and B. D. Ashley.* Dept. of Biochemistry, Army Med. Research Lab., Fort Knox, Ky.

As reported previously, the UV absorption of Δ^4-3-ketosteroids is decreased by the interaction ("binding") with proteins (Arch. Biochem. Dec., 1956). This method was applied to estimate the interaction between α, β-unsaturated C_{19}- and C_{21}- steroid ketones and human serum albumin (HSA) or beta-lactoglobulin (BLG). In general, the 11β-hydroxy group weakened the interaction markedly. The UV-absorption depression by HSA was less with all 11β-hydroxysteroids studied than with their 11-keto counterparts; such differences were not found in the corresponding, generally weaker interactions with BLG. Introduction of a 17 α-hydroxy group did practically not affect the interaction with HSA, weakened however the complexing with BLG. Whereas in the cases tested the introduction of an 11-keto group did not markedly influence the binding to either protein, the simultaneous introduction of 11-keto and 17 α-hydroxy groups decreased the interaction with HSA (cortexone-cortisone). Hydroxylation at C-21 weakened the binding with BLG but did not affect that with HSA. 2-Methyl derivatives showed stronger interaction with HSA than their normal counterparts. Introduction of a Δ^1-double bond in cortisone or cortisol did not influence the absorption depression caused by either protein. Acetylation of the 21-hydroxy group strengthened the interaction. Concerning the responsible groupings in the protein molecule, addition of up to 8 moles of caprylic acid per mole HSA did not change the high absorption-depression of progesterone, suggesting that the interaction does not take place at the anion-binding sites. Blocking the histidyl residues by 20, 40 or 60 $ZnCl_2$ per HSA did not change the decreased light absorption of testosterone.

1152. IN VIVO AVAILABILITY OF AMINO ACIDS FROM RAW AND SEVERELY AUTOCLAVED FRESH PORK TO IMMATURE AND ADULT RATS. Priscilla Wheeler* and Agnes Fay Morgan. Dept. of Home Economics, Univ. of California, Berkeley.

Fifteen amino acids have been determined by quantitative paper chromatography of their dinitrophenyl derivatives in plasma of portal blood taken from immature and adult rats after a fast or at different time intervals after feeding 5 gm of either raw or severely autoclaved fresh pork. Increases in all the amino acids, except ornithine, at 1/2 hr after feeding the meat were strikingly greater in the plasma of the young rats fed the raw meat than in that of those fed the autoclaved meat. For the first 2 hr after feeding the older rats showed a similar but less marked picture. There was also a sex difference in the time interval at which peak values were obtained in the older rats fed raw pork. It is suggested that these results support the view that failure of growth in animals fed severely heated proteins is due to inhibition of hydrolysis of the heated proteins by digestive enzymes.

1153. PROPERTIES OF AN ATYPICAL ASCORBIC OXIDASE OF MYROTHECIUM VERRUCARIA. Gordon A. White* and Frederick G. Smith. Dept. of Botany, Iowa State College, Ames.

Washed mycelial pellets from liquid shake cultures were homogenized with powdered glass in cold buffer (pH 6.3, 0.01 M phosphate-0.005 M citrate). Extracted enzyme was cleared by cold centrifugation at 20,000 X g and stored frozen. Activity was assayed manometrically at 30°, usually in presence of glutathione to avoid autoxidation. Adjustment to pH 4.5 and centrifugation separated soluble and insoluble enzyme fractions which acted independently. Both fractions have optimum pH ranges of 4.2-4.8, but the pH-activity curve of the soluble fraction is skewed to the alkaline side. The pH

optimum is independent of substrate concentration, and at pH 4.5 reaction rates are proportional to enzyme concentration. Both fractions show uptake of 1/2 mole of O_2/mole of ascorbate and K_s values of about 0.0015 M. Reaction kinetics are influenced by what appears to be reaction inactivation. Variation in enzyme-substrate ratios resulted in zero order, first order, or more complicated relationships. Addition of gelatin or serum albumin did not reduce enzyme inactivation. Dialysis showed no evidence of soluble cofactors. Both soluble and insoluble fractions were stimulated by iodoacetate, diethyldithiocarbamate, p-chloromercuribenzoate, cystine and ferricyanide. Ethyl xanthate, BAL, p-benzoquinone, cysteine, phenylthiourea, and 8-hydroxyquinoline were inhibitory. Cyanide and azide inhibited the insoluble fraction but stimulated the soluble fraction. Preliminary evidence indicates substrate specificity for L-isomers. (Supported in part by a contract with the Office of Naval Research.)

1154. PURIFICATION AND CHARACTERIZATION OF AMYLOMALTASE INDUCED IN ESCHERICHIA COLI. Herbert Wiesmeyer* and Melvin Cohn. Dept. of Microbiology, Washington Univ. School of Medicine, St. Louis, Mo.

Maltose induces E. coli to synthesize amylomaltase an enzyme catalyzing the reaction, n maltose $\rightleftharpoons$ n glucose + $(glucose)_n$ (Monod and Torriani. Ann. Inst. Pasteur 78:65, 1950). By means of the spectrophotometric determination of reduced TPN resulting from the phosphorylation and oxidation of glucose by hexokinase and glucose-6-phosphate dehydrogenase, respectively, an assay was developed for the purification of amylomaltase from extracts prepared by sonication. The specific activity of the best preparation is 62.9 μM of glucose liberated/min/mg nitrogen at 28°, pH 6.9. This represents a 400-fold purification over the crude sonicate. Amylomaltase (having an S_{20} of 4.7×10^{-13} sec^{-1}) sediments in the ultracentrifuge as a single major peak. The molecular weight based on an axial ratio of 1.0 was calculated to be 56,000. The enzyme shows maximal activity at pH 6.9. At this pH the K_m for maltose as substrate is 1.1×10^{-3} M. The equilibrium constant, determined at pH 6.9, was found to be 1.5 at 38° based on a polymer length of 4-6 glucose units as determined by Doudoroff et al. (J. Biol. Chem. 179:921, 1949). Investigations of maltose analogs revealed that the following are competitive inhibitors: β-methyl maltoside ($K_I = 2.5 \times 10^{-3}$ M), α-methyl glucoside ($K_I = 8.1 \times 10^{-3}$ M), and α-phenyl glucoside ($K_I = 1.0 \times 10^{-2}$ M). Trehalose, cellobiose, lactose, sucrose, and β-methyl mannoside showed neither an inhibitory action nor substrate activity. These studies form the starting point of an investigation into the mechanism of synthesis of this enzyme.

1155. PROGESTERONE METABOLISM IN EVISCERATED FEMALE RATS BEARING DECIDUOMA. Walter G. Wiest (intr. by Leo T. Samuels). Dept. of Biological Chemistry, Univ. of Utah College of Medicine, Salt Lake City.

Previous reports from this laboratory have demonstrated the metabolic conversion of progesterone to 6 other substances in the eviscerated female rat. One of these has been identified as Δ^4-pregnen-20α-ol-3-one. Subsequent investigation has been directed toward identifying the other metabolites, locating their sites of origin, and correlating their formation with a demonstrable physiological response of progesterone in the animal. Progesterone-4-C^{14} was injected intravenously into eviscerated pseudopregnant rats each bearing deciduoma in the right uterine horn. One hour following administration the 2 horns of the uterus, as well as other organs and tissues, were removed and assayed for radioactivity. Finally the ethylene dichloride soluble extracts of these tissues were chromatographed to determine identity of the radioactive steroids present. Labeled steroids were present in higher concentration in the deciduoma-bearing horns than in the control horns; however, the isotope concentration in the ovaries, kidneys and adrenals was higher than in either uterine horn. The nature of the steroid content varied with the tissue analyzed. No qualitative difference in metabolites was seen between the 2 uterine horns. Analyses of the steroid content of tissues indicate that the dehydrogenases responsible for the reduction of ring A are distributed mainly in kidney and uterus; while the enzymes capable of reducing the side chain are present in fat and muscle. Metabolic patterns similar to those seen in vivo have been obtained from the respective tissues in vitro.

1156. ALTERED REACTIVITY OF PROTOTROPIC GROUPS IN CHYMOTRYPSINOGEN. Philip E. Wilcox (intr. by Walter B. Dandliker). Dept. of Biochemistry, Univ. of Washington, Seattle.

Evidence for large alterations in reactivity of at least 6 prototropic groups in α-chymotrypsinogen (MW 25,000) has been found by chemical analysis and potentiometric titration. Part of the evidence depends upon determination of the numbers of glutamic and aspartic acid residues which are in the form of glutamine and asparagine. A study has been made of the reduction of esterified chymotrypsinogen with lithium borohydride. After reduction, the protein was hydrolyzed and free glutamic and aspartic acids were determined by ion exchange chromatography. The effects of both reduction time and esterification time were explored. Under the chosen conditions, these effects are small. The results indicate that 11 residues of glutamic acid and 15 residues of aspartic acid are unaffected by esterification and reduction. Probably these 26 residues, except perhaps 1 or 2, are present in the protein as glutamine and asparagine; experiments on the release of ammonia by 1 N NaOH, 5 N HCl, and conc. HCl indicate from 23.6-24.3 amide groups. There remain about 10 residues with free carboxyl groups. When these analytical results are compared with titrations of chymotrypsinogen, and guanidinated or acetylated chymotrypsinogen, it becomes evident that the ionizations of 6 prototropic groups have been altered in the native protein and appear below pH 5. Spectrophotometric data on the ionization of tyrosine residues show that 2 of the 4 tyrosine residues have altered reactivity. Titration of guanidinated cymotrypsinogen likewise indicates an alteration of 2 tyrosine residues. Evidence pointing to additional and novel interactions between prototropic groups in chymotrypsinogen will be discussed.

1157. CALCIFIED ATHEROMATOSIS IN THE RAT. George F. Wilgram (intr. by C. C. Lucas). Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Canada.

Wissler et al. and Fillios et al. have shown that atheromatous lesions can be induced in aorta and coronaries of rats. These findings have been confirmed and extended in our laboratory. Young male rats fed a basal diet consisting of 35% egg-yolk, 25% lard, 30% mixed carbohydrates, and supplements of vitamins and minerals, grew particularly well and after 10 months were very obese (average weight 800 gm, maximum 1100 gm). In 5 out of 14 of these

obese rats lipomatous coronary lesions only were encountered after an experimental period of 16 months. However, calcifying atheromatous lesions were found in 3 out of 4 animals after experimental periods which ranged from 8-11 months, when the diet was supplemented with 0.5% cholic acid, 0.2% propylthiouracil and large doses of Viosterol (175 mg %). Two animals showed numerous areas of old and recent cardiac infarcts. The lesions in aorta and coronaries consisted of a degenerated calcified media which was separated from the epithelium by large plaques of calcified debris and subendothelial ground substance, intra- and extracellular masses of lipid and cholesterol crystals in the subintima and organizing fibrous tissue without capillaries. The basal diet supplemented with vitamin "D" alone produced the Moenckeberg-type of vascular disease in aorta and coronaries. No atheromata were encountered. Propylthiouracil and cholic acid supplements without vitamin D produced atheromatous lesions without calcification as described by Fillios. Thirteen animals on the basal diet were exposed to cold (+2°) for periods of 6-14 months. Eight out of 13 of these relatively young animals showed lipomatous lesions comparable to those observed by Malinow in old rats fed a stock diet. Possibly exposure to cold aggravates the "normal" effects of aging. (Supported by the Life Insurance Med. Research Fund.)

1158. PREPARATION AND PROPERTIES OF BIOTINYL PHOSPHATE. Virginia R. Williams and Sally E. Cauthen.* Dept. of Agricultural Chemistry and Biochemistry, Louisiana State Univ., Baton Rouge.

As part of a study of biotin derivatives of possible metabolic significance, biotinyl phosphate was synthesized and tested for biological activity. The biotinyl phosphate was prepared by reacting biotinyl chloride with monosilver dihydrogen phosphate in dimethyl formamide. The excess phosphoric acid was neutralized with anhydrous sodium carbonate and the supernatant solution removed and evaporated to dryness. The biotinyl phosphate was recrystallized from absolute alcohol. The resulting product melted at 120-122°, was chromatographically homogeneous, and was 95% pure by carbon-hydrogen analysis. Confirmatory phosphate and biotin analyses were also made. With aged cell suspensions of Bacterium cadaveris, biotinyl phosphate proved no more effective than biotin in stimulating the deamination of aspartic acid. A study of the hydrolysis behavior of biotinyl phosphate showed it to be stable in acid solution but rapidly hydrolyzed at pH 7.5. This fact alone is sufficient to account for the failure of biotinyl phosphate to exhibit enhanced metabolic activity as compared with biotin.

1159. METABOLISM OF GLUTAMINE IN MAMMALIAN CELLS GROWN IN TISSUE CULTURE. William J. Williams and Lionel A. Manson (intr. by W. C. Stadie). Wistar Inst. of Anatomy and Biology and Univ. of Pennsylvania, Philadelphia.

Glutamine is known to be important for the growth of mammalian cells in tissue culture, and for this reason an investigation of the metabolism of this compound in homogenates of such cells has been undertaken. A human malignant cell, strain HeLa, was grown on glass using a modified Eagle's medium with human serum. The cells were harvested with Versene, and then were homogenized in 0.3 M sucrose by grinding with sand. The homogenates catalyze the conversion of glutamine to glutamic acid and also the formation of an hydroxamic acid from glutamine and hydroxylamine, as indicated by a positive ferric chloride test. The glutaminase activity is localized largely in the mitochondria. Equimolar quantities of glutamic acid and ammonia are formed from glutamine. The reaction is activated by phosphate and thus appears to be similar to that studied by Carter and Greenstein (J. Natl. Cancer Inst. 7:433, 1947). The enzyme system responsible for the formation of an hydroxamic acid from glutamine and hydroxylamine is found largely in the supernatant solution after removal of the subcellular particles by prolonged high-speed centrifugation. The reaction rate is proportional to enzyme concentration. Activity is diminished by dialysis, and thus far attempts to restore activity have been unsuccessful. Thus no evidence for a dissociable cofactor has been obtained to date. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis.)

1160. QUANTITATIVE ASSAY OF INTRINSIC FACTOR ACTIVITY BY URINARY EXCRETION OF RADIOACTIVE VITAMIN B_{12}. William L. Williams and Leon Ellenbogen.* Research Div., American Cyanamid Co., Pearl River, N. Y.

The extent to which 2 or more preparations of intrinsic factor (I.F.) differ in potency is best assessed if they and a reference sample are all tested with a single patient with pernicious anemia. Vitamin B_{12} labeled with Co^{56} (half-life, 72 days) was found to be suitable for consecutive urinary excretion studies in the same patient and to possess distinct advantage over vitamin B_{12} labeled with Co^{60} (half-life, 5.3 hr). The use of repeated flushing doses appeared to have little effect on the response in the urinary excretion test for I.F.; 8 patients were given daily up to 22 flushing doses of 1 mg of unlabeled B_{12} over a period of 36 days and showed essentially the same response to a standard intrinsic factor preparation at the beginning and end of this period. In a series of urinary excretion tests the dosage of a crude sample of intrinsic factor was increased from 45-180 mg, and in a second series with a potent intrinsic factor sample the dosage was increased from 2-30 mg. The excretion of vitamin B_{12} in the test increased with increasing amounts of I.F. indicating that excess of I.F. did not inhibit absorption. There was no relationship between the urinary excretion values obtained with $B_{12}Co^{60}$ alone and the values obtained with $B_{12}Co^{60}$ plus the standard I.F. in a series of tests on 95 pernicious anemia patients. It appears that total rather than net excretion values are to be preferred in evaluating the test. A 2.0 μg oral dose of radioactive B_{12} in the urinary excretion test appeared superior to a 0.5 μg dose since one third of the 95 patients excreted more than 0.17 μg B_{12} thus indicating that they absorbed more than 0.51 μg when given the standard I.F. preparation.

1161. USE OF CYSTINE-S^{35} FOR DETERMINING RATE OF WOUND HEALING. Martin B. Williamson and Harold B. Haley.* Depts. of Biochemistry and Surgery, School of Medicine, Loyola Univ., Chicago, Ill.

The rate of tissue replacement in wounds can be measured, at present, by the rupture strength of the wound or by analysis of the concentration of some constituent of the regenerating wound tissue. Both methods require large numbers of experimental animals, each of which can be used for only one determination. It has been shown that regenerating wound tissue accumulates relatively large amounts of the sulfur-containing amino acids, especially cystine. With this as a basis, attempts to develop a new technique for measuring the rate of healing, utilizing S^{35}

labeled cystine, have been made in which the same animal can be used for repeated determinations. White rats and humans were given a single dose of S^{35} labeled cystine shortly prior to the time of wounding and the radioactivity measured in the wound area and in normal skin, at several intervals after wounding. In order to obtain the same geometrical characteristics for counting and to measure the radioactivity in the same area of skin or regenerating wound, a special gas-flow counter and "reference plate" were devised. It was observed that the concentration of S^{35} in the regenerating wound tissue was about 2-3 times as high as that in normal skin within 4 days after wounding. The level of S^{35} in the wound tissue remained fairly constant or even increased to some extent over a period of about 2 wk, while the S^{35} in the skin decreased during this period. By proper plotting of these data, a curve can be constructed which is indicative of the rate of healing.

1162. ROLE OF CALCIUM AND SULPHYDRYL GROUPS IN PANCREATIC LIPASE. E. D. Wills (intr. by H. B. Pierce). Univ. of Vermont, College of Medicine, Burlington.

The activity of a partially purified preparation of hog pancreatic lipase has been found to be retained after prolonged dialysis against deionised water but to be considerably reduced or completely destroyed by the addition of metal binding substances such as citrate, oxalate or versene. If versene treatment was not prolonged or carried out at low temperatures, the enzyme was reactivated by the addition of calcium or less effectively by strontium, barium or magnesium salts. The stability of lipase to heat is, however, considerably reduced by versene treatment. Thus after 10 min exposure to 50° untreated lipase lost 36% of its activity but it lost 86% in presence of 0.002M versene. Addition of Ca^{++} before exposure to 50° protected the versene treated enzyme but once inactivation had occurred it was irreversible. Lipase is powerfully inhibited by Cu^{++}, Ag^{+} and Hg^{++} but the inhibition may be partially reversed by addition of Ca^{++}. Sr^{++}, Ba^{++} and Mg^{++} were considerably less effective in reversing this inhibition. p-chlormercuribenzoate and neoarsphenamine inhibited lipase but iodoacetate, ferricyanide and alloxan did not inhibit. Lipase partially inhibited by p-chlormercuribenzoate was completely inactivated by addition of Cu^{++} or Hg^{++} in very low concentrations. This fact and the reversal by Ca^{++} suggests that these metals do not inhibit primarily by combination with -SH groups. The function of Ca^{++} and sulphydryl groups will be discussed in relation to the mechanism of lipase action.

1163. REACTIONS OF SELENATE IN THE SULFATE ACTIVATING SYSTEM. Lloyd G. Wilson* and Robert S. Bandurski. Botany Dept., Michigan State Univ., East Lansing.

Recent studies in this laboratory (J. Am. Chem. Soc. Dec., 1956) and that of Robbins and Lipmann (ibid.) have established a two-step reaction mechanism for the enzymatic formation of 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Enzyme I, ATP-sulfurylase, reversibly catalyzes the formation of adenosine-5'-phosphosulfate (APS) and pyrophosphate (PP) from ATP and sulfate. Enzyme II, APS-kinase, in the presence of ATP and APS, catalyzes the formation of PAPS. Selenate, like sulfate, is acted on by the sulfurylase resulting in PP elimination from ATP. Incorporation of P^{32}-labeled PP into ATP in the presence of selenate and the sulfurylase suggests at least transient formation of a nucleotide-selenate compound. Attempts at isolating nucleotide-bound $Se^{75}O_4$ = utilizing the charcoal adsorption technique of Crane and Lipmann (J. Biol. Chem. 201:235, 1953) from reaction mixtures containing an estimated 0.3 μM of nucleotide-bound selenium (based on PP formation) show that less than 0.004 μM survived the incubation and isolation procedure thus indicating the instability of the selenium analogues under the conditions employed. (Supported in part by the Natl. Science Fndn.)

1164. INFLUENCE OF pH ON KINETICS OF LACTIC DEHYDROGENASE REACTION. Alfred D. Winer* and George W. Schwert. Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.

It has been concluded from previous studies (J. Biol. Chem. 223:157, 1956) that the reversible reaction catalyzed by crystalline lactic dehydrogenase of heart muscle can be represented by the reaction sequence:

$$E + O \rightleftharpoons EO$$
$$EO + L \rightleftharpoons EOL \rightleftharpoons ERP \rightleftharpoons ER + P$$
$$ER \rightleftharpoons E + R$$

where E is the enzyme and O, R, L, and P are oxidized and reduced DPN, lactate and pyruvate, respectively. In this sequence, the substrate cannot be bound by the enzyme itself but only by the enzyme-coenzyme complex. Initial reaction velocities have been measured from each side of the reversible reaction over a range of pH from 5.5-10.5 at 28.5° in 0.2 ionic strength buffer. The value of the maximum velocity from the lactate side increases with increasing pH while that from the pyruvate side decreases with increasing pH, but both are relatively insensitive to pH changes. The Michaelis constants for lactate and for DPN have minimal values at about pH 8.5. The Michaelis constant for pyruvate increases markedly with increasing pH while that for DPNH increases slightly. The equilibrium constant for the overall reaction, calculated from the kinetic constants by the method of Alberty, remains essentially constant over this range of pH. The constant characterizing the dissociation of the enzyme-DPN complex is essentially invariant with pH while that characterizing the dissociation of the enzyme-DPNH complex increases sharply above pH 9. The implications of these findings for the mechanism of the reaction will be discussed.

1165. FUNCTION OF VITAMIN A. George Wolf,* S. R. Wagle* and B. Connor Johnson. Div. of Animal Nutrition and Radiocarbon Lab., Univ. of Illinois, Urbana.

It was reported last year that vitamin A is involved in glycogen biosynthesis from acetate. Further evidence showed it to act at some point between the triose and hexose levels, as no difference in radioactivity of glycogen could be found in vitamin A-deficient and pair-fed animals when injected with glucose-C^{14} or fructose-C^{14}, whereas the differences were very large when using two- and three-carbon precursors. Since these differences were not found to occur in glucose synthesis with liver homogenates of vitamin A-deficient and pair-fed rats, it was surmised that vitamin A might function indirectly through adrenal glycotropic hormones. Vitamin A-deficient and pair-fed rats were injected with cortisone acetate (5 mg/day) for 4 days, prior to injection of acetate-C^{14}. The total radioactivity of the glycogen isolated from their livers was almost identical. As was to be expected from previous experiments, no difference was found in glycogen radioactivity, when glucose-C^{14} was injected into vitamin A-deficient and pair-fed, cortisone-treated animals. These experiments

suggest that vitamin A deficiency may influence glucose synthesis indirectly, by causing a disturbance in adrenal hormone function.

1166. SERUM TPN-SPECIFIC ENZYMES IN LIVER DISEASE. S. K. Wolfson, Jr. and H. G. Williams-Ashman (intr. by Paul Talalay). Ben May Lab. for Cancer Research and Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.

Isocitric (ICD) and 6-phosphogluconate (PGD) dehydrogenases are present in human blood serum and erythrocytes. They are completely specific for TPN and can be determined spectrophotometrically. The activity of both ICD and PGD in the sera of apparently healthy individuals is of the same order of magnitude (approximately 120 μM substrate oxidized/cc of serum/hr at 25°). The levels of serum ICD are unchanged in pregnancy, and in patients with acute febrile illness, diabetes, hyperthyroidism, myocardial infarction and neoplastic disease per se. Very high levels were found in patients with certain liver diseases, e.g. homologous serum jaundice, cholangitis, acute hepatitis and neoplastic disease with liver metastases, whereas normal serum ICD levels were found in cirrhosis of the liver. The elevations of serum ICD in liver disease were of a much greater magnitude than corresponding alterations in serum PGD and alkaline phosphatase activity. Great increases in the ICD of rat serum were found after the administration of carbon tetrachloride. Serum aldolase and PGD levels were also increased by this agent, but to a lesser extent than ICD. Serum ICD levels were also increased after bile duct ligation and after partial hepatectomy in the rat, but were unchanged in animals subjected to limb ligation shock.

1167. L-RIBULOSE-PHOSPHATE - D-XYLULOSE-PHOSPHATE STEREOISOMERASE FROM AEROBACTER AEROGENES. M. J. Wolin,* F. J. Simpson* and W. A. Wood. Dept. of Dairy Science, Univ. of Illinois, Urbana.

A stereoisomerase which converts L-ribulose-5-phosphate (L-Ru-5-P) into a compound reacting in two enzymatic tests for xylulose-5-phosphate (Xu-5-P) has been purified from A. aerogenes. After incubating L-Ru-5-P with stereoisomerase, phosphoketopentoepimerase, ATP, and phosphoribulokinase, ADP was determined with phosphoenolpyruvate kinase, lactic dehydrogenase, phosphoenolpyruvate and DPNH, as DPNH disappearance. With a purified stereoisomerase, product formation was determined continuously in the presence of excess transketolase-phosphoriboisomerase, D-ribose-5-phosphate (R-5-P), D-glyceraldehyde-3-phosphate (G-3-P) dehydrogenase, DPN and arsenate, as the rate of DPNH formation. L-Ru-5-P did not react in these systems unless the stereoisomerase was present. R-5-P alone did not reduce DPN in the transketolase test indicating that the stereoisomerase and other enzymes were free of epimerase. When a purified epimerase from bakers' yeast was substituted for the stereoisomerase, D-R-5-P caused DPN reduction but L-Ru-5-P did not. Thus, stereoisomerase and epimerase have been prepared free of each other. Thirty-four μM of L-Ru-5-P incubated with the stereoisomerase yielded 11 μM of D-Xu-5-P. After treatment with phosphatase and deionization, 2 cysteine-carbazole-positive peaks were eluted from Dowex-1-borate. Peak 1 was identified as xylulose and peak 2 as ribulose by paper chromatography and by their behavior in the orcinol test. Since 1) D-Xu-5-P is the only D-ketopentose substrate for transketolase, 2) D-Ru-5-P rather than L-Ru-5-P is required for phosphoribulokinase, 3) an L-pentulose phosphate donor for transketolase would yield L-G-3-P, and 4) L-G-3-P does not yield D-G-3-P in these preparations, it is evident that the stereoisomerase forms D-Xu-5-P from L-Ru-5-P. In the pathway of L-arabinose metabolism which involves D-pentose and D-hexose phosphates, the change from L- to D-configuration can be attributed to stereoisomerase action.

1168. SYNTHESIS OF LACTOSE IN THE COW: DISTRIBUTION OF C^{14} IN GLUCOSE AND GALACTOSE. H. G. Wood, P. Siu* and P. Schambye.* Dept. of Biochemistry, Western Reserve Univ., Cleveland, Ohio and Dept. of Spec. Pathology, Royal Veterinary College, Copenhagen, Denmark.

When $CH_3C^{14}OOH$ was injected intravenously into a cow the glucose and galactose of the milk lactose had the same total activity. In contrast, in the perfused udder experiments 95% of the C^{14} was in the galactose. It is proposed that free glucose is the source of the glucose and hexose phosphates that of the galactose. Acetate-C^{14} administered by vein is converted by the liver to blood glucose-C^{14} which is phosphorylated in the udder; thus both the glucose and galactose moieties acquire activity. When introduced directly into the udder by perfusion, acetate-C^{14} is converted to hexose phosphate but the glucose, which is derived from the unlabeled perfused glucose, remains unlabeled. It has been possible to simulate both the intravenous and perfusion experiments in a single intact cow by injecting acetate-C^{14} directly into one udder artery and collecting milk separately from the injected and non-injected udder halves. Lactose from the injected half contained a high C^{14} activity and 90% of the C^{14} was in the galactose. From the opposite side the activity was low and equal in the 2 hexoses. In addition on the injected side the distribution of C^{14} in the galactose was very unsymmetrical (85% of C^{14} in C-4), resembling a perfusion experiment. The marked asymmetry is believed to result from a slow triose isomerase reaction and from randomization of the C^{14} through the 6-phosphogluconate oxidative pathway in the udder. The glucose of the injected side and the galactose and glucose of the non-injected side had nearly symmetrical C^{14} distribution resembling the liver glycogen and blood glucose. Although the major source of carbon for lactose synthesis is blood glucose, the perfusion or arterial injection permits demonstration of a de novo synthesis from small molecules in the udder per se.

1169. EFFECTS OF INSULIN AND ANTI-INSULINS ON MELANOMA AND BRAIN GLUCOLYSIS IN RELATION TO SODIUM-POTASSIUM BALANCE. Mark Woods. Natl. Cancer Inst., Bethesda, Md.

Anaerobic glucolysis by slices of mouse S91 melanoma and brain in glucose-bicarbonate Krebs-Ringer solution was markedly affected by the exogenous sodium-potassium ratio. $Q^{N_2}_{CO_2}$ of melanoma in KR modified so as to replace all Na^+ with K^+ was only 50-60% of that in an all-Na^+ KR. At either extreme, responses to exogenous insulin were minimal, in part due to lowered endogenous anti-insulin action (Na^+ KR) or to increased endogenous anti-insulin action (K^+ KR). Optimal responses to exogenous insulin (0.3 unit/ml) occurred at intermediate K^+ levels, depending on prior host stress. Whereas increasing the K^+ level accentuated endogenous anti-insulin action, percentage response to exogenous anti-insulin factors (testosterone, DOCA, Podophyllum

extract) was decreased. Aerobic pre-incubation of melanoma slices for 10 min with insulin at 40°C increased subsequent anaerobic insulin effects (7-40°C). The in vitro $Q_{CO_2}^{N_2}$ of mouse brain was more sensitive than melanoma to K^+/Na^+ ratio. In an all-Na^+ KR, or with low K^+ (ca. 0.001M), rate time curves similar to those for insulin-sensitive melanoma were obtained (maximum $Q_{CO_2}^{N_2}$ 4-6 at 20°C and 10-15 at 40°C), and testosterone and Podophyllum extract were likewise markedly inhibitory (40 and 80% respectively at 50 ppm). While sensitivity of tumor glucolysis to these inhibitors is correlated with responsiveness to insulin, brain slices responded very irregularly to insulin, the increases in $Q_{CO_2}^{N_2}$ varying from 0-30%. Insulin responses, and high glucolytic rates, with brain were not previously observed (Biochim. et Biophys. Acta, 12:329, 1953) when high-K^+ media were employed.

1170. IDENTIFICATION OF ESTRADIOL-17β FROM DOGFISH OVA. Herbert H. Wotiz, Charles Botticelli,* Fredrick L. Hisaw Jr.* and Ira Ringler.* Dept. of Biochemistry, Boston Univ. School of Medicine and Biological Labs. Harvard Univ., Cambridge, Mass.

Dogfish ova (836 gm) were extracted with ethanol containing 2% hydrochloric acid. Following neutralization of the extract, the mixture was filtered and evaporated in vacuo leaving an oily residue. The estrogenic activity of this and subsequent fractions was determined by both the Allen-Doisy and Astwood techniques. The oily residue was dissolved in chloroform and the solution extracted with 1 N sodium hydroxide. Following adjustment of the alkaline extract to pH 7, it was reextracted with chloroform. The material obtained was evaporated to dryness and subsequently partitioned by counter-current distribution with 29 transfers (upper phase: 70% methanol - lower phase: 50% chloroformic carbon tetrachloride). The pooled contents of tubes 3-9 and 10-19 contained estrogenic activity. Repartitioning of the 2 fractions in the same system resulted in the appearance of estrogenic activity only in tubes 10-19. The material from these tubes was chromatographed on paper in a toluene-propylene glycol system and the areas corresponding to estradiol-17β and estrone were eluted. The 2 fractions were rechromatographed in the same system along with a mixed chromatogram with authentic estradiol-17β and estrone. Only estradiol-17β was detected by this procedure.

1171. MEVALONIC ACID METABOLISM. Lemuel D. Wright. Grad. School of Nutrition, Cornell Univ., Ithaca, N. Y.

2-C^{14}-Mevalonic acid (beta, delta-dihydroxy-beta-methyl valeric acid) was incubated with various fractions of rat liver homogenate prepared by differential centrifugation. Utilization of the factor was followed by disappearance of microbiological activity as determined by direct assay with Lactobacillus acidophilus 4963 and by the appearance of C^{14} in the non-saponifiable fraction and in cholesterol. When suitably supplemented with ATP, DPN, fructose diphosphate, and versene cell-free (500 x g supernatant) mitochondria-free (9,000 x g supernatant) or microsome-free (105,000 x g supernatant) preparations are highly active in the utilization of mevalonic acid and the conversion of the factor to radioactive non-saponifiable material. The microsomes do not utilize mevalonic acid but are required for the final conversion to cholesterol. The high yields of cholesterol and/or precursors encountered when mevalonic acid rather than acetate is used as a substrate affords a more satisfactory system for a study of the nature of the intermediates involved in the formation of cholesterol. Progress in this direction will be summarized.

1172. BIOSYNTHESIS OF PECTINIC ACID METHYL ESTERS. Pei-Hsing Lin Wu* and Richard U. Byerrum. Kedzie Chem. Lab., Michigan State Univ., East Lansing.

Previous experiments have shown that the methyl group of methionine may be transferred as a unit to form the methyl esters of pectinic acid in radish plant metabolism. It has now been demonstrated that other compounds which contribute to the one-carbon pool may also be introduced into pectinic acid methyl esters. Included in the group of methyl precursors tested were glycine (α-carbon), serine (β-carbon) formate and formaldehyde. After feeding these compounds, labeled with carbon-14, to radish plants, the isolated pectinic acid contained most of its radioactivity in the methyl ester carbon. The results indicate that in the biosynthesis of methyl ester groups in photosynthesizing plants, carbon dioxide is converted to two- or three-carbon compounds which are split to give a one-carbon unit at the oxidation state of formaldehyde. This unit may then either be reduced to the methyl of methionine and introduced into methyl esters by transmethylation, or may be reduced directly without methionine as an intermediate, to form methyl esters.

1173. PASTEUR AND CRABTREE EFFECTS. R. Wu* and E. Racker. Div. of Nutrition and Physiology, Public Health Research Inst., New York City.

Measurements of glycolytic enzymes in ascites tumor cells show that their activities could support a more rapid rate of glucose utilization than that found with intact cells. Determinations of the intracellular concentration of adenine nucleotides, hexose phosphates and inorganic orthophosphate (P_i) under various conditions point to P_i as the limiting factor in glycolysis and respiration. The addition of glucose to washed cells results in a marked fall in intracellular P_i, as well as in the respiration rate. Iodoacetate lowers the level of P_i further and decreases acid soluble adenine nucleotides. This could account for the persistence of the Crabtree effect (inhibition of endogenous respiration by glucose) even in the presence of iodoacetate. Tumor cells (10 mg protein) utilize 6 μM of glucose aerobically in 30 min. With iodoacetate, which does not inhibit respiration, only 1.5 μM of glucose are utilized and accumulate mostly as fructose-1, 6-diphosphate. With dinitrophenol (or anaerobically), in spite of a lowering of the total intracellular ATP, 13 μM of glucose are taken up. With iodoacetate and dinitrophenol present together, practically no glucose disappears. These data indicate that glycolytically produced ATP is used more efficiently for glucose phosphorylation than ATP generated in mitochondria. Since dinitrophenol alone increases both glucose utilization and P_i levels, and since the P_i level aerobically is well below saturation of tumor glyceraldehyde-3-phosphate dehydrogenase, it is proposed that the glycolytic generation of ATP is limited by the availability of P_i. An explanation of the Pasteur effect based on the decreased production of glycolytic ATP and the lack of availability of mitochondrial ATP will be presented.

1174. EFFECT OF CITRATE ON SOLUBILITY OF TRICALCIUM PHOSPHATE IN URINE. Claude L. Yarbro (intr. by James C. Andrews). Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill.

The calcium complexing ability of citrate has considerable implication in the treatment and prevention of urinary calculi, especially in view of the reports in the literature concerning the lowered citrate excretion in chronic stone formers. For these reasons the effect of added citrate upon the solubility of tricalcium phosphate in urine was investigated. Citrate in the amounts of 0, 1, 2 or 5 gm/l. was added to urine and the urine equilibrated for 6 hr with solid tricalcium phosphate, 2 gm/100 ml. These equilibriations were repeated following adjustment of the pH of the urine to pH's of approximately 5, 6, 7 and 8. At periodic intervals of time aliquots were taken for phosphate analysis. Comparison of the changes in phosphate concentration following equilibration at the various pH levels indicates that the calcium complexing ability of urinary constituents is minimal in the range of pH's from 6-7. This effect appears to be caused by constituents in urine other than citrate. Comparison of the changes in phosphate concentration following equilibration of urines containing added citrate with those to which no citrate was added shows that the effect of citrate upon calcium phosphate solubility in urine is only slightly decreased with increasing pH of the equilibration media. (Supported by Grant A-248 from the Natl. Inst. of Arthritis and Metabolic Diseases, PHS.)

1175. FORMATION AND HYDROLYSIS OF α-GLUTAMYLHYDRAZIDE. Allan S. Yard,* J. H. Watherby and Herbert McKennis, Jr. Dept. of Pharmacology, Med. College of Virginia, Richmond.

Using aqueous extracts of acetone powders from liver (rabbit and pigeon) and kidney (rabbit and hog) together with glutamine, hydrazine and cofactors, evidence has been obtained for the formation of α-glutamylhydrazide. This, together with previous data (Speck; Schou, Grossowicz, Tajtho and Waelsch), suggests a wide species capability for synthesis of the compound. Disappearance of hydrazine from enzyme solutions was followed by the p-dimethylaminobenzaldehyde reaction. Metabolized hydrazine was recovered upon acid hydrolysis. α-glutamylhydrazide administered to the dog was excreted in part as hydrazine as evidenced by formation of benzalazine when benzaldehyde was added to the urine of treated animals. The hydrazide directly or by way of its metabolites produces fatty livers and elevates blood ammonia values.

1176. PROPERTIES OF PURIFIED TYROSINASE AND HEMOCYANIN. K. Yasunobu,* L. C. G. Thomson* and H. S. Mason. Univ. of Oregon Med. School, Portland.

During study of enzymic oxygen transfer, properties of purified mushroom tyrosinase and hemocyanin (Cancer magister) were determined. Hemocyanin sedimented at 105,000 gm,95-98% homogeneous: $s_{20,w}$ = 25.0 x 10^{-13}(0.15 M phosphate, pH 6.5); $D_{20,w}$ = 2.27 x 10^{-7} (0.15 M phosphate, pH 6.5); partial specific volume, 0.74; electrophoretic mobility, -5.1±0.2 x 10^{-5}; m. w., 950,000; Cu = 0.17%. Upon removal of Cu by anaerobic dialysis against cyanide, a protein with the same ultracentrifugal properties was obtained. Its sulfhydryl content was determined by amperometric titration against silver in Tris buffer (method of Benesch, Lardy and Benesch). For every 24 copper atoms removed from hemocyanin, 6 sulfhydryl groups were revealed. The protein contains at least 11 N-terminal aspartate residues per molecule. Tyrosinase purified by a modification of the method of Keilin and Mann was electrophoretically homogeneous at pH 8.6 (barbiturate) and 5.0 (acetate), and ultracentrifugally homogeneous at pH 7.0 (phosphate). Cu = 0.2%; 3200 catecholase units/mg dry weight; catecholase/cresolase ratio = 230 - 250.

1177. ENZYME SYNTHESIS IN THE COTYLEDONS OF GERMINATING SEEDS. J. Lowell Young (intr. by F. E. Deatherage). Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

It is well known that the activities of certain enzymes present in seeds increase during germination. Experiments were designed to determine whether these increased activities are the result of net enzyme synthesis or of enzyme activation. Results indicate that both possibilities occur. The ATP-ase activity of the cotyledons of germinating peas increases 10- to 15-fold during the first 5 days of germination. This increase can be prevented by DNP and by protein synthesis inhibitors (chloramphenicol, p-fluorophenylalanine) without preventing germination. The ATP-ase activity in the cotyledons also fails to increase when the growing parts of the seed are dissected out. This may be a result of the fact that the mitochondria of these cotyledons are not capable of oxidative phosphorylation (Vanecko. Plant Physiol. 31:XXIV, 1956). These results indicate that the large increase in ATP-ase activity in the cotyledons is due to net enzyme synthesis. In contrast, the changes in proteolytic activity of pea cotyledons do not appear to be affected by protein synthesis inhibitors or by dissecting out the growing parts of the pea seed.

1178. INCORPORATION OF C^{14}-ATP INTO SOLUBLE RNA ISOLATED FROM 105,000 x g SUPERNATANT OF RAT LIVER. Paul C. Zamecnik, Mary L. Stephenson,* Jesse F. Scott* and Mahlon B. Hoagland. J. C. Warren Labs., Huntington Hosp. of Harvard, at Massachusetts General Hosp., Boston, Mass.

Previous work from this laboratory indicates that a pH 5.2 precipitate of the 105,000 x g supernatant of a rat liver homogenate contains enzymes which are able to activate amino acids. At these meetings Hoagland et al. report that the same enzyme preparation contains RNA (S-RNA) which becomes labeled with C^{14}-leucine upon incubation with the C^{14}-amino acid and ATP. Parallel experiments using ATP-8-C indicate that the C^{14}-mononucleotide becomes incorporated into the RNA of this same fraction. There is no incorporation of C^{14}-ATP into the microsomes incubated alone. When the pH 5.2 enzymes were incubated with microsomes and C^{14}-ATP, upon recentrifugation, the bulk of the counts were found in the RNA of the pH 5.2 enzymes and not in the microsome RNA. The incorporation is most rapid in the first 10 min. The labeling of S-RNA is proportional to the enzyme concentration up to 6.0 mg protein/ml and proportional to the concentration of ATP up to a saturation level of 0.01M ATP. About 0.04 μM ATP are incorporated/mg S-RNA during a 20' incubation at 37°C. C^{14}-ADP is incorporated at one quarter the rate of C^{14}-ATP. The reaction is inhibited by ribonuclease. Alkaline digestion of the labeled RNA releases about 75% of the counts as the nucleoside, while only 5% each were recovered as 2' and 3' adenylic acids. This indicates that the incorporated adenylic acid is in a terminal position and not distributed throughout the chain.

1179. INTRODUCTION OF 5-BROMOURACIL INTO DNA OF E. COLI. Stephen Zamenhof, Kenneth Rich*

and Rosalie De Giovanni.* Dept. of Biochemistry, Columbia Univ., New York City.

We have reported (S. Zamenhof et al. J. Bacteriol. 71:60, 1956) that upon growing in 100 μg of 5-bromouracil/ml, the thymine-requiring strain I of E. coli replaces 48% of thymine in DNA by 5-bromouracil, whereas wild strain B - only 4.4%. This study has now been extended to various growth conditions and strains. The large replacement of thymine by 5-bromouracil is not a characteristic of the parent strain (Roepke) from which I originated: another mutant of this strain requiring phenylalanine replaces only 7.5% even on 200 μg of 5-bromouracil/ml. On the other hand, a mutant of this strain requiring uracil when grown under similar conditions replaces 18%, presumably because such mutant is a partial thymine-requirer. We now find that the wild strain B becomes a partial thymine- (or thymidine-) requirer when grown in 25 μg to 1 mg aminopterin/ml; in 250 μg aminopterin and 200 μg 5-bromouracil/ml, 14% of thymine in the DNA of this strain becomes replaced by 5-bromouracil. By increasing the concentration of 5-bromouracil to 2 mg/ml, the wild strain B can be forced to replace 18% of thymine in DNA by 5-bromouracil even when no aminopterin is present. However, 6 mg of 5-bromouracil/ml causes high mortality in 24 hr; such cells were found to contain no 5-bromouracil in DNA.

1180. DPN-LINKED OXIDATION OF AMINO ACIDS BY ENZYMES FROM TOBACCO LEAVES. Israel Zelitch. Biochemical Lab., Connecticut Agricultural Exp. Station, New Haven.

Crude enzyme preparations obtained by the fractionation of clarified tobacco leaf sap and exhaustive dialysis have been found to oxidize a number of amino acids as evidenced by the reduction of 2,6-dichlorophenolindophenol. The Thunberg technique was used. Of the amino acids tested, the highest rate of dye reduction is observed with L-glutamic acid, L-aspartic acid, L-glutamine, L-asparagine and glycine. In order to observed reduction of the dye in the presence of any of these amino acids, it is also necessary that DPN be added. A DPN-specific glutamic acid dehydrogenase can also be demonstrated by direct methods in these preparations by the formation of DPNH at 340 mμ. Furthermore DPNH is formed when glutamine is the substrate. Although the reduction of DPN has not yet been demonstrated in this manner for the other amino acids mentioned, the available evidence suggests that DPN-linked amino acid dehydrogenases analogous to glutamic acid dehydrogenase are also present.

1181. REACTIVE SITE OF AMINE OXIDASES. E. Albert Zeller, J. Barsky, L. A. Blanksma and J. C. Lazanas. Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.

The reactive site of amine oxidases has been studied with various methods, e.g. by establishing a detailed substrate and inhibitor pattern employing more than 200 compounds, by computing affinity constants from kinetic data, and by developing non-enzymic models. It was recognized that monoamine oxidase (MO) and diamine oxidase (DO) share many properties. Both attack monoamines as well as diamines and both are blocked by monosubstituted alkyl- and arylhydrazines. These substances can be viewed as isosteric amines. Among the substrates of MO, histamine, 1-Methyl-4-β-aminoethyl) imidazole, and 1-Methyl-5-β-aminoethyl) imidazole are found, among DO's substrates tyramine and mescaline. Monoacylhydrazides and N,N'-diacylhydrazides are inhibitors of DO only. Eight pairs of optically active hydrazides, including 7 pairs of amino acid hydrazides were tested; in each case one antipode turned out to be more active than the other by approximately one pi-unit. The reactive surface of DO, therefore, possesses an asymmetric structure, consisting probably of L-amino acids. In considering the influence of substitutions on the efficiency of inhibitors and substrates, the active area of amine oxidases must measure several square Angstrom units. Short aliphatic monoamines are bound by both amine oxidases in a similar fashion. With the introduction of a second amino group, the ability to be degraded by MO drops markedly, while the affinity for DO increases tenfold. The second amino group does not react with DO by virtue of its positive charge but by its nucleophilic nature.

1182. NATURE OF LINKAGE OF PYRIDINO-PROTEINS TO ELECTRON TRANSPORT SYSTEM. D. M. Ziegler and A. W. Linnane (intr. by C. A. Elvehjem). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

The present work is concerned with the linkages by which the pyridino-protein enzymes are bound to the electron transport system in phosphorylating beef heart mitochondria (BHM). Oxidative phosphorylation is abolished whenever the BHM are fragmented by any procedure which leads to the solubilization of the pyridino-proteins. Mechanical damage to the BHM, induced by a high speed Virtis blendor, in the presence of phosphate leads to essentially complete solubilization of the malic and isocitric dehydrogenases. The α-ketoglutaric, glutamic and pyruvic dehydrogenases are solubilized but to a lesser extent. In the absence of phosphate during the blending a large portion of the pyridino-protein dehydrogenases remain associated with the particulate fraction. BHM have been disintegrated by a variety of procedures to determine some of the factors governing the association of pyridino-protein enzymes with the fragmented particles.

1183. ULTRAVIOLET INHIBITION OF SUCROSE SYNTHESIS IN PLANTS. L. P. Zill (intr. by W. J. Van Wagtendonk). Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

It has been found that the mechanism of sucrose synthesis in the wheat plant is more sensitive to photochemical inhibition by ultraviolet light than is the initial fixation of carbon dioxide (Zill and Tolbert. Plant Physiol 30:XIV, 1955). On the basis of Sinsheimer's (Rad. Research 1:505, 1954) demonstration of photochemical instability of the uridylic acids and the demonstrated involvement of uridine diphosphate glucose (UDPG) in sucrose synthesis by plants (Cardini, Leloir, and Chiriboga. J. Biol. Chem. 214: 149, 1955) the effect of ultraviolet light on UDPG has been investigated. At a dose of 35 ergs/sq mm/sec of ultraviolet light, predominately at 2537 Å, an exponential destruction of the absorption at 260 mμ was observed with a half-life of approximately 5 min. This half-life is of the same order of magnitude as the inhibition of sucrose synthesis in the plant upon exposure to ultraviolet light of the same intensity. This evidence supports, but does not prove, the destruction of UDPG by ultraviolet light as the basis for inhibition of sucrose synthesis in the green plant. Uridine diphosphate and triphosphate were found to exhibit an almost identical sensitivity to ultraviolet light as did UDPG. The loss of absorption at 260 mμ could, in all compounds studied, be reversed by addition of acid to a pH of 1, similar to Sinsheimer's observations with the uridylic acids.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Forty-seventh Annual Meeting

CHICAGO, ILLINOIS, APRIL 15-19, 1957

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1184. EFFECTS OF INTERNEURON DEPRESSANTS ON INHIBITION AND FACILITATION OF THE PATELLAR REFLEX. D. H. Abdulian,* W. R. Martin* and K. R. Unna. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

Recent work (Eccles, 1956) has indicated that ipsilateral inhibition of the patellar reflex may involve an interneurone. For this reason, it was decided to reinvestigate the effects of interneurone depressants on spinal inhibition. The patellar reflex was elicited once every second with a tapping hammer in the unanesthetized spinal cat and was recorded on an ink writing kymograph. The central ends of cut ipsilateral and contralateral sciatic nerves where stimulated for a 5 sec interval every minute with graded stimuli. The degree of facilitation and inhibition was expressed as percentage of the mean control patellar reflex height and this value was plotted against stimulus strength on arithmetic graph paper. The stimulus-response curves thus obtained were linear. The effects of mephenesin, 6-methyl-2-amio-benzothiazole, pentobarbital and meprobromate on the stimulus response curves of both inhibition and facilitation were studied. All of the above mentioned drugs elevated the threshold voltage for inhibition and facilitation. As the animal recovered from the effects of the drug, the stimulus response curves for inhibition and facilitation returned to their original positions. These drugs also altered the slope of both the inhibitory and facilitatory stimulus response curves in that any increment of voltage produced less facilitation and inhibition following the drug. The elevation of thresholds for facilitation by these drugs was greater than the rise in threshold of inhibition; however, with small doses this difference was not striking. These results indicate that ipsilateral inhibition of the patellar reflex is vulnerable to the drugs employed.

1185. METABOLIC FATE OF ACETAZOLEAMID S^{35} (DIAMOX) IN THE CAT. L. B. Achor* and L. J. Roth. Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

The urine of cats dosed with 150 mg/kg acetazoleamide (including S^{35}-labeled drug) by i.p. route was collected after 45 min and analyzed for the presence of possible transformation products. Extraction of urine with water-saturated methyl ethyl ketone removed all S^{35}-containing material into the organic phase. Analysis of such extracts by paper chromatography in conjunction with ninhydrin visualization and radioautography revealed the presence of unchanged drug only. Comparison of acetazoleamide isolated from both paper strips and cellulose columns with authentic material served to establish the identity of the unchanged drug.

1186. EFFECT OF CORTISONE ON THE ISOLATED PERFUSED RABBIT HEART; RELATION OF POTASSIUM. Adeleke Adeyemo,* Gilbert Allen* and Walter M. Booker. Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.

We have shown previously that cortisone, hydrocortisone and 9-alphaflurohydrocortisone when administered at constant intervals to the isolated perfused heart of the rabbit cause, after an initial positive inotropic effect, a reduction in stroke, decrease in rate and in rhythm, resulting in subsequent failure. In the cortisone experiments we showed that when hearts were perfused with Tyrode's solution that had a diminished amount of potassium in it, the hearts were more sensitive to the "cortisone effect." When, on the other hand, the potassium concentration in perfusion fluid was increased, the hearts were more resistant to the "cortisone effect." We suspected that perhaps cortisone was having its effect partly by removal of potassium from the cells. In the present experiments we have been able to restore the heart action, in some instances, to normal by the administration of potassium. Also, we can greatly lower the effect of potassium on the heart, when administered originally by subsequently administering cortisone. Experiments are now underway in which ratios are being established between cortisone and potassium and joint administrations made.

1187. DEPRESSOR ACTION OF RESERPINE. R. P. Ahlquist. Dept. of Pharmacology, Med. College of Georgia, Augusta.

It is commonly accepted that a substance in order to merit consideration as an antihypertensive agent should produce a relatively sustained hypotension when administered i.v. to anesthetized dogs. We have found that reserpine, a widely used antihypertensive, in many cases did not produce hypotension in anesthetized dogs even when very large doses were administered. A decade of experience with a standardized anesthesia (morphine, 10 mg/kg s.c. followedin 30 min with pentobarbital 15 mg/kg, i.v.) has demonstrated that such dogs have an arterial pressure of about 100 mm Hg. Reserpine in doses up to 20 mg/kg did not significantly alter the arterial pressure, nor did it diminish the characteristic responses to

acetylcholine, epinephrine, levarterenol, histamine, vasopressin or isoproterenol. However, bradycardia was produced. The results were consistent with the ideas that reserpine has no direct peripheral depressor action.

1188. APPARENT ISOTONICITY OF CELL FLUID IN BOILED TISSUES. Johannes W. Appelboom (intr. by Peter K. Knoefel). Inst. for Med. Research and Dept. of Pediatrics and Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.

The osmotic activity of quickly frozen crushed tissues was hypertonic to plasma. Such hypertonicity could have been due to rapid metabolism, or to physical changes in the proteins. Therefore experiments were designed to measure tissue-osmotic activity after stopping metabolic processes by rapid heating. Bits of tissue, quickly removed from anesthetized dogs, were transferred to small beakers containing water kept at 98°C. After 30 min at 98°, data showed evidence of diffusion equilibrium between ambient and tissue fluids. Freezing point depression of the undiluted tissue was calculated from that determined on the diluted supernatants together with the appropriate weights of beakers, water and dry tissue. Whole blood, liver and kidney were slightly hypotonic to serum by 20, 10 and 10 mOs/l. respectively. Such differences could have been caused by losses of CO_2 during boiling. This was confirmed by analysis for CO_2 content of boiled and fresh tissues. Losses of CO_2 in whole blood, liver and kidney were approximately 15, 8 and 14 mM/kg, respectively. Adding these values for CO_2 loss to that of tissue osmotic activity, the freezing point depression of such tissue extracts was practically equal to that of plasma. Control experiments on the changes in activity coefficients of tissue electrolytes with dilution showed that the difference of osmotic activity between boiled tissue and plasma was no more than minus 3.0%. These data would indicate that tissues are isotonic with plasma, if it is valid to extrapolate the results to conditions present in the living cell. (Supported by Public Health Service Grant A-461, and by Army Contract No. DA-49-007-MD-481.)

1189. ENZYMATIC SYNTHESES OF N-GLUCURONIC ACID CONJUGATES. Julius Axelrod, Joseph K. Inscoe* and Gordon H. Tomkins.* Nat. Inst. of Mental Health and Nat. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

An enzyme which can transfer glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to the nitrogen of a number of amines (aniline, p-phenetidine p-toluidine) has been found. Only the microsomes of mammalian liver showed enzyme activity. The enzymatically formed N-glucuronides were readily hydrolyzed in an acid medium. However, in the presence of β-glucuronidase the rate of hydrolysis of the N-conjugates were no greater than that observed with the boiled β-glucuronidase. Preliminary evidence suggests that a single enzyme is involved in the formation of ether, ester and N-glucuronide linkages since it was shown that regardless of the substrate used (phenol, carboxylic acid or amine) the pH optima, rates of inactivation and intracellular localization of the enzyme that synthesizes glucuronides were similar. That an amino compound can react with UDPGA to give an N-glucuronic acid conjugate is consistent with the previous findings that other nucleophilic (electron donating) reagents such as alcohols, phenols and carboxylic acids can attack the electrophilic anomeric carbon atom of UDPGA. Since the glycosidic bond of UDPGA has an α configuration it would appear that the enzymatic synthesis proceeds by a backside attack to form β-glucosiduronic acids.

1190. EFFECT OF COMBINED TREATMENT WITH PLASMIN (FIBRINOLYSIN) AND HEPARIN ON THE LYSIS OF CLOTS IN VARIOUS STAGES OF ORGANIZATION. N. Back,* J. L. Ambrus and Irving B. Mink.* Roswell Park Mem. Insti. and Univ. of Buffalo, Buffalo, N. Y.

It has been shown in previous studies (Circulation Res. 4:430, 440, 1956) that plasmin is able to lyse fresh clots produced in dogs with I^{131} labeled fibrinogen. As organization proceeded response to plasmin therapy decreased (Fed. Proc. 15:396. 1956), often several days after dissolution of the radioactive clot, reformation of a new nonradioactive clot was observed. This was occasionally seen even in animals receiving plasmin daily. Inability of plasmin to dissolve these newly formed clots was explained by the gradual development of resistance to plasmin. Heparin treatment given after plasmin therapy prevented reformation. Heparin given simultaneously with plasmin resulted in increased effectivity and toxity. Heparin alone promoted spontaneous lysis of fresh clots. Various treatment schedules of plasmin-heparin combinations were compared against clots in various stages of organization.

1191. PHYSIOLOGICAL DISPOSITION OF CHLOROTHIAZIDE (6-CHLORO-7-SULFAMYL-1,2,4-BENZOTHIADIAZINE-1,1-DIOXIDE), A SALURETIC-DIURETIC AGENT. John E. Baer, Lorraine Leidy and Antonia V. Brooks (intr. by Karl H. Beyer). Merck Inst. for Therapeutic Research, West Point, Pa.

Chlorothiazide is an inhibitor of carbonic anhydrase whose spectrum of renal activity in the dog resembles that of mercurial diuretic agents, especially with respect to causing an increase in the excretion of sodium chloride. Beyer, Baer, Russo and Haimback (Fed. Proc., 1957) have reported that the agent has a duration of action of less than 5 hr and that it has remarkably little tendency to produce metabolic acidosis upon repeated administration. These differences from the behavior of acetazolamide made it desirable to study the renal excretion and fate of chlorothiazide. When 10 mg/kg was given intravenously to 2 dogs, 67 and 76% of the dose was excreted within 1 hr and 86 and 99% was excreted in 6 hr, the plasma level decreasing with corresponding rapidity. The renal clearance of chlorothiazide was shown to exceed glomerular filtration rate in experiments wherein the plasma concentration of chlorothiazide was maintained by venocylsis. Probenecid, at a dose that completely inhibits tubular secretion of penicillin, partially depressed the renal tubular secretion of chlorothiazide. Four hours following the intravenous administration of 20 mg/kg of 3-C^{14}-labeled chlorothiazide to a bilaterally nephrectomized dog, the plasma level was 26.3 mg/l. and the erythrocyte/plasma concentration ratio was 0.7 Some 40% of the dose was in the bile and another 10% in the intestinal tract. Small amounts of chlorothiazide were found in liver, lung and spleen, and little or none in muscle, fat, brain or cerebrospinal fluid. A colorimetric method for chlorothiazide has been developed and is based upon the diazotization and coupling of an alkaline hydrolysis product. Results of chemical and

radioactive assays are in agreement and there is no present evidence for transformation of chlorothiazide in the dog.

1192. RELATIONSHIP BETWEEN BLOOD PRESSURE AND THE COMMON CAROTID OCCLUSION PRESSOR RESPONSE WITH A CRITICAL ANALYSIS OF PREVIOUS METHODS. Walter Baird, Harold F. Hardman, Bert K. B. Lum and Daniel Suits (intr. by Maurice Seevers). Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

The relationship between carotid occlusion pressor response and existing blood pressure was shown to be linear within the blood pressure limits of 70 and 160 mm Hg (Prochnik et al. Am. J. Physiol. 162:553, 1950). We were unable to verify the linearity of this relationship below a blood pressure value of 100 mm Hg. Our data indicate that the pressor response approaches zero when the blood pressure falls below 100 mm Hg. These results are observed in animals anesthetized with sodium pentobarbital when blood pressure declined spontaneously, was reduced by hemorrhage or was lowered through the use of a vasodilator substance (sodium nitroprusside). We calculated the regression line slope for each individual dog and averaged these values. In this way each dog made an equal contribution to the average slope value. In order to validate the observation that the pressor response to carotid occlusion approached zero at 100 mm Hg, a series of dogs was subjected to stepwise hemorrhage. The pressor response to carotid occlusion was evoked at several intervals, allowing time for blood pressure stabilization, between the initial blood pressure and the disappearance of the pressor response. The observed values at which the pressor response disappeared show a high degree of correlation with the predicted values derived from the regression line. A discussion of the use of this method in evaluating ataraxic drugs will be presented.

1193. ACTION OF SOME ANTICHOLINESTERASES ON ELECTRICAL AND MECHANICAL EVENTS IN THE ISOLATED HEART. W. W. Baker and J. McPhillips.* Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.

Some investigators have assigned an important role to the cholinesterases of the heart in the regulation of contractility, rate and rhythmicity. Preliminary results suggested that the anticholinesterases varied qualitatively and quantitatively in their actions on the heart. Using a system previously described, the electrical and mechanical events of the isolated frog heart were recorded simultaneously during perfusion with the anticholinesterases. In general, the anticholinesterases influenced contractility more readily than the rate but had less pronounced effects on the electrogram and rhythmicity of the heart. Effective concentrations of the agents varied between $5x10^{-4}$ and 10^{-6}. Physostigmine and TEPP depressed the amplitude and rate. However, physostigmine inverted the T-wave: whereas, TEPP had little effect even at higher concentrations. Arrhythmias developed occasionally with TEPP. At the concentrations employed, DFP initially decreased and then slightly increased the force of contraction with only minor changes in the electrogram. Meostigmine increased both the amplitude and rate along with an increase of the negativity of the T-wave. This action was demonstrated after TEPP and DFP and in the presence of physostigmine. The effect of neostigmine on the isolated heart appears to be similar to the stimulation noted in skeletal muscle following the inhibition of cholinesterase. Although the effect is direct and unrelated to the inhibition of cholinesterase, the receptors involved appear to be cholinergic since atropine antagonized neostigmine.

1194. ANTAGONISM OF SOME ACTIONS OF TETRAETHYLPYROPHOSPHATE BY MORIN. N. M. Balotin* and J. M. Coon. Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.

Since most of the acetylcholine which accumulates during poisoning by cholinesterase inhibitors arises from its continuous synthesis, a depression of this synthesis would be a logical approach to the treatment of such poisoning. The flavonoid compound morin (2', 3, 4', 5, 7-pentahydroxy flavone), shown in vitro to suppress acetylcholine formation (Beiler et al. Arch. Biochem. 26:72, 1950), was used in studies on animals and isolated tissues poisoned with the anticholinesterase tetraethylpyrophosphate (TEPP). Muscle fasciculations in mice, induced by sublethal doses of TEPP, were inhibited by morin. The LD_{50} of TEPP in atropinized mice was increased about 30% when 50 mg/kg of morin was administered prophylactically. In atropinized rats under anesthesia, morin increased the tolerance to repeated small doses of TEPP administered at 5-min intervals. The peristaltic-type rhythm induced by TEPP in segments of isolated rabbit ileum was reversed to the control pendular rhythm by morin, but no antagonism was evidenced toward the action of acetylcholine on this tissue. TEPP and physostigmine, in concentrations which inhibited contractions of the isolated frog heart, were completely antagonized by morin. Acetylcholine, however, in concentrations which merely depressed the contractions of the frog heart were not antagonized by morin. These findings are compatible with, but do not prove, the idea that morin prevents or reverses certain effects of TEPP by reducing acetylcholine synthesis. In any case an atropine-like mechanism is not involved. The level of protection afforded by morin in TEPP-poisoned animals is of theoretical interest but not of practical value.

1195. EFFECT OF HYPOPHYSECTOMY ON PLOIDY DISTRIBUTION IN RAT LIVER. Allan D. Bass, Clara E. Dunn.* Pharmacology Dept., Vanderbilt Univ. School of Medicine, Nashville, Tenn.

It has been reported previously from this laboratory (Bass, McArdle and Grisham. Proc. Soc. Exper. Biol. & Med.; 89:7, 1955) that following hypophysectomy of 140-gm rats there is an increase in diploid nuclei in the liver. DiStefano and Diermeier (Proc. Soc. Exper. Biol. & Med. 92:590, 1956) using young rats (98 gm) reported that the shifts in ploidy following hypophysectomy could be due to an arrest in mitotic activity. It was desirable, therefore, to further investigate the effects of hypophysectomy under conditions of similar dietary intake. 140 gm hypophysectomized and normal rats were pair fed for 21 days and the results compared with ad lib fed control animals. At the beginning of the experiment biopsy samples of liver tissue were obtained in the 2 groups of control animals for comparison with the liver samples taken 21 days later. No shifts in ploidy were observed in these groups over a 21-day period. The percentage of diploid nuclei in the livers of the rats pair-fed, fed ad lib and hypophysectomized were: 16.8+S.E. 1.072, 17.6+S.E. 1.033, 35.4+S.E. 0.974,

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respectively; indicating that hypophysectomy in adult rats results in a shift toward diploid nuclei.

1196. POSSIBLE ROLE OF POTASSIUM IMBALANCE IN THE CARDIAC ACTIONS OF OUABAIN. P. Bass* and K. I. Melville. Dept. of Pharmacology, McGill Univ., Montreal, Canada.

The possible significance of potassium imbalance in the actions of ouabain on the isolated perfused hearts of rabbits has been investigated. Concomitant changes in coronary inflow, heart contractions and electrograms were recorded concurrently by methods previously described. These were correlated with net changes in potassium movement, as determined by analyses of the potassium concentrations in the perfusing fluids and those in the perfusates collected at short intervals (1 min) during continuous perfusions with varying concentrations of ouabain added to, either normal Locke or Locke containing decreased or increased potassium. In 6 experiments, with ouabain (7.5 mcg/ml) normal Locke, initial coronary constriction followed by definite coronary dilatation ensued; associated with characteristic initial sinus slowing followed by increased contractions, tachycardia, extrasystoles, A-V block and final complete arrest (av. 18.3 min)· and concomitant with initial increased uptake of potassium followed by progressive loss. In 6 similar experiments with reduced potassium (3 with potassium-free Locke) there was similar initial coronary constriction followed by slight dilatation; associated with less marked stimulation as well as T wave heightening, tachycardia and extrasystoles - final cardiac arrest ensuing more rapidly (av. 9.6 min). Terminal ventricular fibrillation observed with potassium-free Locke without ouabain did not occur. In 3 similar experiments, using double potassium concentrations, only prolonged coronary constriction ensued until cardiac arrest, whereupon, changing to normal Locke, marked immediate coronary dilatation and cardiac stimulation occurred; little or no cardiac stimulation was observed concomitantly, but there was marked QRS widening followed by low voltage complexes with final arrest (av. 20.3 min). Only minimal potassium imbalance was observed in these 2 latter groups of experiments. The overall findings would rather suggest that potassium imbalance per se is not a primary factor in the direct cardiac actions of ouabain. The possible significance of other ionic changes is being studied. (Supported by a grant from the Nat. Research Council of Canada.)

1197. PLACEBO AND NON-REACTORS TO ANALGESICS. Robert C. Batterman. Dept. of Medicine, New York Med. College, Flower and Fifth Ave. Hosps., New York City.

Analysis of 417 patients with chronic musculoskeletal pain treated from 3 to 70 months (majority over 12 months) with 2-52 trials of drug evaluation (majority over 8 trials) reveals that 3 categories according to analgesic effectiveness are possible. Ten percent of patients are nonreactors and do not respond to any medication regardless of dosage and duration of therapy. Thirteen percent always respond to any medication and are therefore true placebo reactors. Seventy-six percent are normal reactors and may respond or not at various times to effective analgesics or placebo medication. Variability of response is characteristic of the majority of patients regardless of diagnosis, severity of pain, medication, dosage and duration of therapy. Two hundred twenty patients were observed for placebo medication. Regardless of diagnosis, severity of pain, age, sex and race, 40% had satisfactory analgesia. On repeat trial in 86 patients, although the same overall effectiveness was achieved, there was considerable change within the group itself. Alteration of response from satisfactory to nonsatisfactory or vice versa occurred in 58%. Exclusion of these patients as placebo reactors on initial testing is therefore erroneous. These patients may not be true placebo reactors and merely reflect the variability of response to any medication. The nonreactors should be excluded from any analgesic program. A method for detection is discussed.

1198. EFFECT OF NOREPINEPHRINE ON CARDIAC OUTPUT AND RELATED HEMODYNAMICS. David P. Baumann and Russell Moores (intr. by L. D. Seager). Dept. of Physiology and Pharmacology, Univ. of Arkansas Med. Ctr., Little Rock.

Recent work in this laboratory has indicated the necessity of revising concepts of the effect of norepinephrine on various hemodynamic factors. Previous opinion has stated that norepinephrine does not change cardiac output demonstrably and causes an increase in blood pressure primarily through a raised peripheral resistance. The effect of norepinephrine was studied in anesthetized mongrel dogs. Cardiac output was determined by the Fick method with the sampling catheter in the right ventricle or pulmonary artery. While a slow i.v. saline drip was maintained a control cardiac output and femoral arterial pressure was obtained. Norepinephrine was then given in doses of 0.1 to 0.6μg/kg min with identical data determined. In 8 of 9 dogs an increase in cardiac output of 10-59% was observed. Administration of norepinephrine after atropine 1.0 mg/kg increased the cardiac output above the levels obtained with norepinephrine alone. This was attributed primarily to an increase in cardiac rate. In 3 of the animals there was no increase in peripheral resistance suggesting that the marked positive inotropic action may at times account for the increase in blood pressure with norepinephrine rather than increased peripheral resistance alone. Left ventricular work was frequently more than doubled. Calculated on a weight basis the increase in cardiac output with norepinephrine was significant.($P < .02 > .01$).

1199. DIFFERENTIATION BETWEEN EFFECTS OF EXOGENOUS AND ENDOGENOUS CATECHOLAMINES ON MYOCARDIAL ELECTROLYTE SHIFTS. E. Bay and I. Bay (intr. by S. Krop). Dept. of Pharmacology, Tulane Univ. School of Medicine, New Orleans, La.

Recent strong evidence indicates that the sympathetic mediator substance is norepinephrine and that the myocardium is able to store this neurohormone in active and/or inactive form. Because of differences in action between injected and endogenously liberated norepinephrine the following experiments were undertaken. Open chest experiments were conducted on 16 bilaterally vagotomized dogs under barbiturate anesthesia. In 1 group of dogs l-norepinephrine was infused at a constant rate of 1.5 μg/kg/min; in another group the anterior ansa subclavia or the sympathetic chain was stimulated, using such an intensity as would give similar cardiac effects as the above described infusion. Arterial and coronary sinus blood samples were taken for K and Na determination. A positive A-V difference was interpreted as uptake, a negative as release of these ions from the myocardium. After l-norepinephrine infusions tachycardia, systemic hypertension and increase in coronary sinus outflow and in ventricular contractile force were observed. In no case were there any measurable K or Na A-V

differences. After stimulation, a cardiac response similar to that from infused l-norepinephrine was found. An increase in contractile force above 30% its control level resulted in a positive A-V difference. No Na shift was detectable. Graded stimulation showed a possible correlation between percentage force increase and amount of myocardial K uptake within certain limits. The above results were reproducible after severing all nerve connections to the stellate ganglion except those to the heart. In 1/3 of the dogs we were able to block up to 70% of the ventricular force increase and the K A-V difference with dihydroergotamine. (Supported by the Tulane Med. School and American Heart Assoc., Inc.)

1200. ANTIFIBRILLARY EFFECTS OF GLYCINE IN HYPOTHERMIA. W. R. Beavers and B. G. Covino (intr. by M. B. Slomka). Arctic Aeromed. Lab., APO 731, Seattle, Wash.

Ventricular fibrillation is the greatest hazard in accidental and surgical hypothermia. Numerous attempts have been made to uncover a means of preventing this lethal cardiac dysfunction. Glycine has been used experimentally to inhibit cyclopropane-epinephrine arrhythmias. Thus the efficacy of glycine as an antifibrillary agent in hypothermia, with and without surgical insult, was studied. Twenty-five dogs, unacclimatized to cold, were anesthetized with pentobarbital and subjected to immersion hypothermia. Twenty-four of the animals succumbed to ventricular fibrillation at a rectal temperature of 23.5-14.5°C. Ten dogs, also unacclimatized, were treated with intravenous glycine (5% solution, 2 gm/kg) and all terminated in asystole at a rectal temperature of 18.5-15°C without exhibiting cardiac arrhythmias. Six dogs receiving glycine were cooled to 18°C rectal and subjected to right thoracotomy, right ventriculotomy and 20-min occlusion of superior and inferior vena cavae. Three animals survived the procedure; 3 fibrillated. No attempt at rewarming was made. Thoracotomy, ventriculotomy and 10 min occlusion of both vena cavae was performed on 8 dogs receiving glycine (5% solution, 0.8 mg/kg) at a rectal temperature of 24°C. Seven were successfully rewarmed.

1201. EPINEPHRINE, ADRENALECTOMY, AND TRAUMA INDUCED DECREASE IN MOUSE LIVER NPSH. Lyle V. Beck and Virginia D. Rieck*. Dept. of Physiology and Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

Register and Bartlett (Science 120:109) have reported that large amounts of epinephrine induce a decrease in rat liver nonprotein sulfhydryl (NPSH) and have interpreted this finding to mean that a stress induced decrease in rat liver NPSH is due to endogenous epinephrine released in response to the stress. In experiments performed in this laboratory it was found that the decrease in male mouse liver NPSH occurring in association with administration of epinephrine in isotonic saline or oil, even at very high dose levels, was little greater than that occurring in association with injection of the vehicle only. Even if all the decrease found were attributed to epinephrine, this epinephrine attributed decrease would still be appreciably smaller than that found by us to occur in mice subjected to severe cold or trauma, or injected with a toxic bacterial polysaccharide or pharmacological doses of insulin. Female mice which had been adrenalectomized and were therefore presumably incapable of releasing endogenous epinephrine, exhibited as great a trauma induced decrease in liver NPSH as did either control or sham operated mice. It is concluded, therefore, that endogenously released epinephrine is not responsible to any great extent for at least some forms of stress induced decrease in mouse liver NPSH. (This research was supported in part by Natl. Insts. of Health Grant-in-Aid RG-3740(C2 & C3).

1202. ANALGESIC EFFECT AND SIDE EFFECT LIABILITY OF DIHYDROCODEINE, CODEINE AND MORPHINE IN MAN. Henry K. Beecher, Joachim S. Gravenstein,* Donald P. Pederson* and Gene M. Smith.* Anesthesia Lab., Harvard Med. School at Massachusetts General Hosp., Boston.

In a double blind study dihydrocodeine, 60 mg and morphine, 10 mg/70 kg body weight, were tested as to their analgesic power in postoperative patients with severe and moderate wound pain. Morphine gave longer lasting pain relief. Sixty milligrams of dihydrocodeine were as good as morphine 45 min after medication, but lost in effectiveness quickly and were significantly weaker than the morphine standard 150 min after medication. This was true for both severe and moderate pain. In a study on physical and psychologic side effects in normal volunteers 60 mg codeine and placebo were studied together with 10 mg of morphine and 60 mg of dihydrocodeine. Depression of respiratory minute volume was marked with morphine, 10 mg, less pronounced with codeine, 60 mg and dihydrocodeine, 60 mg and absent with placebo. Psychologic tests of mood and sensation were administered to these subjects before and after medication with the listed drugs and information concerning sedation, mental clouding, anxiety, hostility and dysphoria were obtained as well as data on dizziness and nausea. Dihydrocodeine, 60 mg, produced more physical and psychologic side effects than dihydrocodeine, 30 mg, which was studied in an earlier investigation.

1203. EFFECTS OF EPHEDRINE AND VERATRAMINE UPON HEART RATE IN THE DOG HEART-LUNG PREPARATION UNDER A-V NODAL RHYTHM. J. M. Benforado, J. Dorner and J. Fuentes (intr. by Otto Krayer). Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

A-V nodal rhythm was established in the dog heart-lung preparation (HLP) by crushing the S-A node. The resulting mean A-V nodal rate was 45 beats/min (11 exps.; range 19-68). Krayer and Benforado (Pfluger's Arch. ges. Physiol. 260:177, 1955) have reported the mean S-A rate of the dog HLP at 38-39°C as 141.7+ 12.8 (S.D.) beats/min. Ephedrine, in doses up to 3 mg, maximally increased the A-V nodal rate to 201 beats/min (range 176-231). The maximum acceleration was 154 beats/min (range 104-192) and the ED_{50} for acceleration was between 0.1-0.2 mg. Comparable values under S-A nodal rhythm (HLP) are: maximal rate, 240 beats/min (range 211-275); maximal acceleration, 106 beats/min (range 90-130) and an ED_{50} of 0.20 mg (Krayer and Ourisson. J. Pharmacol. and Exper. Therap. 112:341, 1954). Under A-V nodal rhythm, the antiaccelerator effect of veratramine following ephedrine is not marked. Dose-response studies could not be carried beyond 40% inhibition of acceleration since, in this dose range, cardiac failure ensued. The I_{40} was about 40 mg which compares with an I_{50}, under S-A rhythm (HLP) of 0.28 mg (Krayer and Ourisson. J. Pharmacol. & Exper. Therap. 112:341, 1954). In 3 experiments, veratramine administered alone, resulted in decreases in A-V nodal rate and 0.3 mg elicited periodic activity. This phenomenon was also observed in some experiments with prior ephedrine administration. It

was not noted in the dog HLP under S-A rhythm (Krayer et al. J. Pharmacol. & Exper. Therap. 113: 446, 1955). In the dog HLP, the A-V and S-A nodes show similar qualitative and quantitative responses to ephedrine. The responses to veratramine differ markedly.

1204. CHLOROTHIAZIDE (6-CHLORO-7-SULFAMYL-1,2,4-BENZOTHIADIAZINE-1,1-DIOXIDE): THE ENHANCEMENT OF SODIUM CHLORIDE EXCRETION. Karl H. Beyer, John E. Baer,* Horace F. Russo* and Audrey S. Haimbach.* Merck Inst. for Therapeutic Research, West Point, Pa.

Whereas mercurial diuretic agents have a greater clinical potency and a more favorable spectrum of action on sodium chloride and water excretion, the (non-mercurial) carbonic anhydrase inhibitor, acetazolamide, has enjoyed wide acceptance in spite of its propensity to produce metabolic acidosis and a substantial excretion of potassium and bicarbonate ions. Chlorothiazide appears to combine uniquely certain attributes of both classes of compounds. It is about 30 times as potent a carbonic anhydrase inhibitor as is acetazolamide in vitro; yet it induces a large and nearly equimolar excretion of sodium and chloride ions without producing a marked acidosis or alkalosis. An average of 4.8 (control), 12.7 and 19.4 mEq of sodium and 5.0 (control), 13.7 and 19.7 mEq of chloride were excreted in 4 hr by 6 dogs after the administration of no drug, 1.0 mg/kg and 3.0 mg/kg of chlorothiazide, respectively. Excretion rates of 100-200 micro-equivalents/min for sodium and chloride were maximal at 1/2-1 hr (at 3.0 mg/kg i.v.) or at 1-2 hr (at 5.0 mg/kg orally) and returned to control values within 5 hr. The effect on potassium excretion at these doses was slight and urinary pH was not necessarily increased. With increased dosage both potassium and bicarbonate excretion (as judged by urinary pH) increased. Even though chlorothiazide was active orally as a saluretic agent at 1.0 mg/kg, the oral administration of 100 mg/kg/day for 4 days did not produce an appreciable effect on blood acid-base balance in the dog. The oral administration of 40 mg/kg/day did not affect the blood electrolyte composition over a period of the 20 wk that such measurements were made. Presumably the secondary attributes of chlorothiazide contribute to its safety (Baer, Leidy and Brooks. Fed. Proc. 1957).

1205. TOXIC EFFECTS OF CARDIAC GLYCOSIDES AND ANTIDOTAL EFFECTS OF DIFFERENT COMPOUNDS IN THE CAT. Rudolf P. Bircher and Suhayl J. Jabbur (intr. by S. C. Wang). Sandoz Pharmaceuticals, Hanover, N. J. and College of Physicians and Surgeons, Columbia Univ., New York City.

In order to study the problem of counteracting toxic effects of cardiac glycosides on the inotropic and chronotropic heart properties and the ECG, procainamide, UR-28 (L-methyl-4-piperidyl-benzamide-HCl), BOL-148 (2-brom-lysergic-acid-diethylamide), and potassium chloride were given in various concentrations simultaneously with digitoxin and Desacetyllanatoside C (Cedilanid D). Both glycosides in concentration of 1:50,000 were infused into the femoral vein at a constant rate of 1 ml/min. Femoral arterial pressure and ECG were continuously recorded with Sanborn apparatus. The lethal doses of digitoxin were 0.400 mg/kg±15% and of Cedilanid D, 0.268 mg/kg±15%. As it was previously reported (Rothlin and Suter, 1947; Rothlin and Bircher, 1954) both glycosides produced with 40-80% of the lethal dose, ST changes, tachycardia and ectopic rhythm. Procainamide in concentrations up to 3 mg/kg/min simultaneously given with these glycosides did not influence the signs of progressing intoxication and death of the cats. Similar results were encountered with UR-28, a potent antifibrillatory compound protecting cats against auricular fibrillation due to electrical stimulation of the auricles (Cerletti, personal communication, 1956). One of the most potent serotonin antagonists, BOL-148 (Cerletti and Rothlin, 1955), in doses of 0.01 mg/min was ineffective. Potassium chloride in concentrations of 0.01 mEq/kg/min was ineffective, but concentrations of 0.03-0.04 mEq/kg/min, in most cases, delayed the appearance of the toxic effects and increased the LD_{100} of both glycosides.

1206. EFFECT OF SEROTONIN ON WATER AND ELECTROLYTE EXCRETION. William P. Blackmore (intr. by Andres Goth). Dept. of Pharmacology, Univ. of Texas, Southwestern Med. School, Dallas.

It has been reported that administration of 1-2 mg of serotonin to patients may be associated with sodium retention and suggested that electrolyte changes following Rauwolfia administration may be related to this activity of serotonin. However, the mechanism whereby this retention occurred has not been explained. In addition, there is still considerable controversy regarding the role of serotonin in the kidney. The objectives of this study were to recheck the action of serotonin on the kidney employing a dose that did not significantly alter blood pressure and to determine the effect of serotonin on sodium excretion and its mechanism of action. Experiments were performed on trained unanesthetized dogs. Glomerular filtration rate and renal plasma flow were measured by the usual techniques. Sodium and potassium were determined with a flame photometer. Following a control period, serotonin base in a dose of 5 μg/kg/min was infused for 1 hr. Studies were then continued for 1 or 2 hr after serotonin infusion. Results obtained indicate no significant change in glomerular filtration rate and only a moderate increase in renal plasma flow. In the majority of the experiments the urine flow was increased above control values during serotonin infusion and reached a maximum during the 1st hr following cessation of the drug. At the end of the 2nd hr it had returned to control values. In all experiments there was a marked decrease in sodium excretion during serotonin infusion which returned to control values or above after cessation of the infusion. These results indicate that infusion of serotonin in the dose employed in dogs does decrease sodium excretion. It is conceivable that a small, although not significant decrease in glomerular filtration rate could account for this change rather than a direct tubular effect. Experiments are in progress in an attempt to elucidate more precisely the mechanism of decreased sodium excretion.

1207. ALTERATION OF TOXICITY OF PARATHION BY ULTRAVIOLET LIGHT. Jane R. Blake,* John P. Frawley, J. William Cook* and O. Garth Fitzhugh. Divs. of Pharmacology and Food, Food and Drug. Admin., Dept. of Health, Education and Welfare, Washington, D. C.

Cook has reported that exposure of Parathion to ultraviolet light produces a product with increased in vitro anticholinesterase properties. Similar conversion could be associated with commercial use; i.e., the material appearing as residue on food might possess a greater toxicity than the parent Parathion. To check this point a sample of technical Parathion was exposed to ultraviolet light until its in vitro anticholinesterase

activity had increased about 4-fold. At that time analysis by the Averell-Norris chemical method showed that only 65% of the original Parathion remained. A comparative subacute toxicity experiment was conducted with this sample and a sample of unexposed Parathion. Four groups of 5 male and 5 female rats were maintained on control diet for 4 wk to permit determination of 5 pretreatment whole blood cholinesterase values by a micromodification of the electrometric method of Michel. Dietary levels of 2 and 5 ppm of exposed and unexposed Parathion were fed for several weeks and then increased to 10 and 20 ppm. Blood samples were drawn weekly from each animal and the cholinesterase values compared with the pretreatment mean of the same animal. To produce comparable lowering of blood cholinesterase it was necessary to feed diets with twice as much exposed Parathion as unexposed. Thus the in vivo anticholinesterase activity correlates well with the Averell-Norris chemical method but not with the in vitro anticholinesterase activity.

1208. EFFECT OF ORALLY ADMINISTERED CORN OIL ON THE DISTRIBUTION OF THIOPENTAL IN VARIOUS TISSUES OF THE RAT. Joan C. Blaskovics* and Wallace D. Winters. Dept. of Pharmacology, Marquette Med. School, Milwaukee, Wis.

Anderson and Magee found that corn oil decreased the duration of anesthesia in rats by 50%. They correlated this with the presence of chylomicrons in the blood of corn oil fed rats and postulated that thiopental was absorbed by these chylomicrons. The purpose of this investigation was to determine the thiopental concentration in blood and other tissues of rats. Male Sprague-Dawley rats were given corn oil orally and injected i.v. with thiopental 2 hr later; when the righting reflex returned they were killed by decapitation. Whole blood, brain, liver and visceral fat were collected and the stomach and intestinal contents flushed out and assayed for thiopental concentration. A 40% decrease in sleeping time was observed in the corn oil group. The concentration of thiopental in the brain, liver and stomach of both groups was not significantly different. The fat and whole blood of the corn oil group contained a lower concentration of thiopental than the control group; a higher concentration than the controls would have been expected in whole blood according to the chylomicron concept. There was a 3-fold increase in thiopental concentration in the small intestine of the corn oil group. (Supported by Grant B-912 of the Natl. Insts. of Neurological Diseases and Blindness, Natl. Insts. of Health PHS.)

1209. TREMOR PRODUCING DRUG 1, 4 DI-PYRROLIDINO-2-BUTYNE ("TREMORINE"). L. E. Blockus* and G. M. Everett. Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.

The further study of Tremorine with a large number of blocking agents and centrally acting drugs has shown the highly specific antagonism of Tremorine tremors by the antiparkinson drugs. The tremor in rats was measured by means of an electronic transducer. The frequency was 8-10/sec. The tremor is present both at rest and during movement but varies in amplitude. Mice show a marked drop of body temperature with doses of 20 mg/kg Tremorine subcutaneously. Over a period of 2 hrs this drop may be as great as 10°C at normal room temperature (25°C). No piloerection is observed. The fall in temperature is rapidly reversed by atropine. The tremor is not to be confused with shivering. Mice placed in an oven maintained at 38°C show no drop in body temperature but show full tremor symptoms. Tremorine-treated mice when placed on a cold copper tray do not show piloerection and show shivering imposed on the tremor. In rabbits the electrocorticogram showed an activated record (low fast activity) but otherwise no changes and no spiking were observed. Tremorine has no anticonvulsant action and causes no change in threshold. Given i.v. the drug causes temporary fall in blood pressure which is blocked by atropine and vagatomy. In isolated gut, 5 gamma produces contraction which is blocked by atropine.

1210. EVALUATION OF ANTISPASMODICS BY AN IN VIVO TEST IN MICE. Harold Blumberg, Hyman B. Dayton* and Samuel M. Gordon.* Research Labs., Endo Labs. Inc., Richmond Hill, N. Y.

An in vivo test has been devised to evaluate the magnitude and duration of antispasmodic or anticholinergic activity on the mouse intestine after oral or parenteral administration. In the oral test fasted mice were fed the antispasmodic by stomach tube, anesthetized 15 min later with sodium pentobarbital i.p. and injected s.c. 15 min afterward with methacholine chloride at 2 mg/kg. After 1 min the peritoneal cavity was exposed. The spasmodic reaction of the small intestine was then observed for 4 min and graded as follows: complete protection - no intestinal contraction or only slight contraction just below the pylorus; partial protection - occasional weak, scattered contractions; and no protection - frequent strong, generalized contractions. Reference curves were made for atropine sulfate, 10 mice being used for each level. In a comparative study the dosages required to give complete protection in 50% of the animals were: atropine sulfate 54±5 mg/kg and homatropine methylbromide 1110±154 mg/kg. Duration of action was determined by injection of methacholine at longer intervals than the usual 30 min after oral administration of the antispasmodic, e.g., 45 and 60 min, with pentobarbital being injected 15 min before the methacholine. Parallel reduction of antispasmodic activity with increasing time interval indicated that duration of action was the same for both compounds at equivalent antispasmodic dosages. Testing by subcutaneous administration differed only in that the antispasmodic was injected at the same time as the pentobarbital, i.e., 15 min before the methacholine. Homatropine methylbromode was approximately 1/8 as active as atropine sulfate subcutaneously.

1211. COMPARISON OF PLASMA TRIGLYCERIDE ESTERASES AND CHOLINESTERASE IN VARIOUS SPECIES. Herbert Blumenthal* and Geoffrey Woodard. Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

In an effort to obtain additional indices for transferring animal toxicity data to man, we have instituted an investigation of the comparative magnitudes from species to species of readily accessible enzyme systems known to be important in drug action, metabolism or detoxication. The organic phosphate insecticides are known to inhibit cholinesterase and there have been reports that the chlorinated insecticides inhibit lipase. Since we have been concerned with the toxicity of these compounds and since both lipase and cholinesterase represent a similar type of activity, we have investigated, initially, plasma cholinesterase, tributyrin and triacetin lipase in various animal species. Measurements have been done manometrically using a conventional Warburg apparatus. Preliminary results indicate that dogs show a cholinesterase activity several

magnitudes higher than rats or rabbits. In the case of plasma lipase activity, on the other hand, the relationship is reversed.

1212. COMPARISON OF CENTRAL ACTIONS OF COCAINE AND LSD. Donald F. Bogdanski, Sydney Spector (intr. by Bernard B. Brodie). Lab. of Chem. Pharmacology, Natl. Heart Inst., Bethesda, Md.

Cocaine injected i.v. into dogs and rabbits in doses of 0.5-2 mg/kg evoked excitation and an increase in sympathetic activity similar to that induced by LSD, as shown by increase in blood pressure, tachycardia, mydriasis, retraction of the nictitating membrane, hyperthermia and hyperglycemia. These effects of cocaine were antagonized by the administration of chlorpromazine and could largely be blocked by spinal section. The data are in accord with the concept that cocaine produces sympathetic predominance mainly by stimulation of the central sympathetic system, in contrast to chlorpromazine which lowers sympathetic activity centrally. Thus, in animals, cocaine, like LSD, produces excitation and sympathetic predominance of central origin which can be antagonized by chlorpromazine. In man, both LSD and cocaine are known to produce psychic manifestations.

1213. ALTERATIONS IN RAT BRAIN 5-HYDROXYTRYPTAMINE LEVELS OF ANTIEPILEPTIC DRUGS. D. D. Bonnycastle, M. K. Paasonen* and N. J. Giarman. Dept. of Pharmacology, Yale Univ., New Haven, Conn.

Up to the present time few substances have been found which will alter the levels of 5-hydroxytryptamine of brain or other tissues. Iproniazid and 5-hydroxytryptophan raise tissue levels, while reserpine and rescinnamine and 11-demethoxyreserpine have been found to cause a depletion of tissue stores. Earlier it was reported by the authors that diphenylhydantoin led to an increase in brain levels of 5-hydroxytryptamine in the rat. This work has been extended and it has been observed that a number of other antiepileptic compounds, including such drugs as Trimethadione, Paramethadione, Phenacetamide, Primidone, Milontin, Phenobartibal and sodium bromide all have this action. It has been shown that the effect upon 5-hydroxytryptamine levels is restricted to the cerebral tissues. The mechanism of the change is under study.

1214. EFFECT OF CONCENTRATION ON ORAL TOXICITY OF DRUGS. J. L. Borowitz,* G. K. W. Yim,* T. S. Miya and C. J. Carr. Pharmacology Dept., Purdue College of Pharmacy, Lafayette, Ind.

Cooper's report that 2 oral doses of 5 ml of water/100 gm of body weight sensitized rats to the oral toxic effects of aminopyrine (J. Am. Pharm. A., Scient. Ed. 45:163, 1956) was confirmed; the oral LD_{50}'s of water-treated and non-water-treated animals were found to be 790 and 1400 mg/kg, respectively. The water given 1 hr prior to the drug was found to be ineffective, whereas water given with the drug enhanced toxicity. The oral water treatment, however, did not affect the i.p. toxicity of aminopyrine. These studies indicated that dilution of the dose facilitated absorption of the drug. The oral toxicities of sodium salicylate, sodium pentobarbital and atropine sulfate were also enhanced by dilution of the dose. The effect was also noted in mice. Dilute and concentrated solutions of 400 mg/kg sodium salicylate were given to rats and the resulting plasma levels determined colorimetrically 10, 30, 60 and 120 min after administration. The average plasma levels of the animals given the dilute solution (0.8%) were found to be approximately 1/4 times greater (approximately 12 mg % higher) than those of rats given the concentrated solution (25%). These findings are contrary to the popular notion that drugs are absorbed more rapidly from concentrated than dilute solutions.

1215. EFFECT OF BLOOD pH ON BARBITURATE SLEEPING TIME IN MICE. Joseph F. Borzelleca (intr. by N. B. Dreyer). Dept. of Pharmacology, Woman's Med. College, Philadelphia, Pa.

Altering the urinary excretion of barbiturates has been shown to influence the duration of sleeping time. Experiments were conducted to determine the effects of alteration of blood pH on barbiturate excretion. Acidosis or alkalosis was induced by the i.p. administration of acetazoleamide (Diamox), 1.0 mg/kg in a 5% solution or sodium bicarbonate, 2.0 gm/kg in a 10% solution 30 min prior to the administration of the various barbiturates. The pH of the blood was determined and found to be within the ranges reported in the literature. An average of 20 male, Swiss-Webster mice per group were used. All barbiturates were administered i.p. in a volume of 0.2 cc/20 gm body weight. Sleeping time was the time between the loss and the return of the righting reflex. The excreted barbiturates were studied chromatographically and colorimetrically. With Pentothal sodium, 50 mg/kg, the control sleeping time was 69 min; in alkalotic mice, 147 min; and in acidotic mice, 247 min. The sleeping times obtained with pentobarbital sodium, 30 mg/kg, were: control, 59 min; alkalotic, 72 min; and acidotic, 76 min. With phenobarbital sodium, 120 mg/kg: control, 213 min; alkalotic, 301 min; and acidotic, 459 min; and with barbital sodium, 200 mg/kg: control, 142 min; alkalotic, 180 min; and acidotic, 286 min. The effects of pH changes on barbiturate excretion and the influence of this on the duration of sleeping time will be considered.

1216. LIPID AND WATER LEVELS IN THE MESENTERY OF TUMOR-BEARING ANIMALS. Eldon M. Boyd and Harold E. W. Binhammer.* Dept. of Pharmacology, Queen's Univ., Kingston, Canada.

The mesentery of albino rats bearing Walker carcinosarcoma 256 was found to lose weight at a rate greater than that of host carcass (host minus tumor). The shrunken mesentery of the tumor-bearing rats contained lower absolute amounts of total lipid, total fatty acids, neutral fat, total cholesterol, free cholesterol, phospholipid, water and nonlipid dry weight, than were present in mesentery of nontumor-bearing twins. The level of neutral fat, expressed as gm/100 gm nonlipid dry weight, was lowered but the levels of phospholipid, total cholesterol, free cholesterol and water, per unit nonlipid dry weight, were very markedly increased in mesentery of the tumor-bearers over levels in nontumor-bearers. The shifts in levels of lipids and water were greater than those seen in the whole carcass (minus tumor) of the host rat. The shifts in mesentery occurred in animals bearing tumors which were 20-40% of host weight with no appreciable correlation with further tumor growth. By elimination, it was concluded that the shifts in levels of lipids and water occurred mostly in the connective tissue of mesentery. This indicated, in turn, the probability that shifts in levels of lipids and water in host carcass are due mostly to shifts in skeletal muscle and connective tissue.

1217. DEPENDENCE OF BILIARY BSP EXCRETION ON ROUTE OF ADMINISTRATION (HEPATIC

ARTERY OR PORTAL VEIN). R. W. Brauer, Otto S. Shill* and J. S. Krebs.* U.S. Naval Radiological Defense Lab., San Francisco, Cal.

Evidence has recently been presented by Andrews Macgraith and Richards that there are differences in handling by the dog liver of the dye sulfobromophthalein sodium (BSP), depending on whether administration is by way of the portal vein or hepatic artery. These experiments have been extended by the use of S^{35}-labeled BSP. Simultaneous injection of equal small doses of labeled and unlabeled dye via the arterial and portal venous routes and analysis of common duct bile, showed that the portal venous route results in faster excretion though total recovery and conversion to the biliary BSP derivatives in independent of route of administration. Using elevated O_2 pressures (2.5 atm.) to arterialize the portal venous supply the observed differences are minimized, suggesting metabolic rather than anatomical factors as the basis of the observed differences. These experiments are interpreted as furnishing no support for the view that BSP excretion is accomplished primarily by the bile duct tissues supplied by the peribiliary arterial plexus.

1218. COMPARATIVE STUDY OF PIGEON EMETIC METHOD AND PROPOSED CHEMICAL METHODS FOR ASSAY OF RAUWOLFIA. H. A. Braun and L. M. Lusky.* Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

Assays have been completed on 10 Rauwolfia samples by the biological and chemical assays. Species of Rauwolfia examined consisted of samples of R. serpentina, R.canescens, R.vomitoria and R.birsuta. The chemical methods used were the chromatographic and the nitrite methods as proposed by Banes and Carol. In the pigeon emesis method dilutions of Rauwolfia were injected into the alar vein. Emesis occurred in from 25 min to 4 hr. Ten pigeons were used on each of 3 doses. A standard was prepared from 5 different lots of R.serpentina and all results obtained were compared with this standard. Generally, in the case of the samples of R.serpentina, there was good agreement between the results obtained by the methods utilized. However, in 1 out of 6 Rauwolfia serpentina samples examined, the potency obtained with the biological methods was more than 125% of that found by the chemical procedures. The advantage of the biological method is that it measures the activity of the alkaloids of all species of Rauwolfia.

1219. INFLUENCE OF HUMAN CHORIONIC GONADOTROPIN ON ANDROGEN SECRETION BY THE TESTIS. Truls Brinck-Johnsen and Kristen B. Eik-Nes (intr. by Leo T. Samuels). Dept. of Biological Chemistry, Univ. of Utah College of Medicine, Salt Lake City.

From the experiments of earlier workers, the following information is available: testosterone and 4-androstene-3,17-dione have been isolated from spermatic venous blood of the dog; histologically, Chorionic Gonadotropin (HCG) has an effect similar to that of Interstitial Cell Stimulating Hormone on both testes and secondary sex organs; finally, in vitro studies show that the presence of HCG can markedly increase the amount of acetate carbon incorporated into testosterone by the testis. In this investigation, we studied the influence of i.v. HCG on the secretion of testosterone and 4-androstene-3,17-dione by the testis of intact nembutalized dogs. The effect was measured by determining the amounts of the 2 androgens in successive 30 min collections of spermatic venous blood from 1 testis. These androgens were isolated by ether extraction and paper chromatography and measured by spectrophotometric and isotope dilution methods. The compounds were characterized by chromatographic and radioactive tracer techniques. Single i.v. injections of 8 IU (or more) HCG/kg body weight caused a significant increase in the amounts of testosterone and androstenedione in the spermatic venous blood collected during the first 1/2 hr thereafter. A maximal level of these androgenic hormones was reached within the 1st hr and was maintained for several hours without additional injections of the tropic hormone. These studies show that HCG has a rapid and persistent effect on the secretion of androgens by the testis of the dog which precedes significant hyperplasia of the interstitial cells.

1220. PYRIDINE-2-ALDOXIME METHIODIDE (PAM) AS AN EFFECTIVE ADJUNCT TO ATROPINE IN THE TREATMENT OF SARIN AND TABUN POISONING. Robert V. Brown, Anne M. Kunkel,* Lea M. Somers* and J. Henry Wills. Chem. Warfare Labs., Army Chem. Ctr., Md.

Cats or dogs, under pentobarbital, injected i.v. with approximately 10 LD_{50} of either sarin or tabun became apneic in less than 1 min. In cats, both chemicals produced complete neuromuscular block within 5 min. In dogs, twitch height was little affected by tabun, but was markedly augmented by sarin. Dogs, like cats, could not maintain a 30-sec tetanus after either tabun or sarin. Atropine sulfate, 0.5 mg/kg, i.v., was usually adequate to start and sustain respiration for 3 hr after sarin poisoning. After tabun poisoning, respiration was started by atropine sulfate, 0.5 mg/kg, i.v., in all dogs, but only 2 of 9 breathed for as long as 1 hr. PAM, 5 mg/kg, i.v., brought about rapid recovery of twitch height and ability to sustain tetanus in both cats and dogs poisoned with sarin; PAM produced recovery more slowly and less completely after tabun. PAM, 5 mg/kg, after atropine sulfate, 0.5 mg/kg, improved breathing in either sarin or tabun poisoning; all of 6 dogs treated with this drug combination breathed for 3 hr after either sarin or tabun. In cats given atropine sulfate, 0.5 mg/kg, before poisoning with sarin, breathing returned in 22±12 min without PAM therapy; with PAM, breathing returned in 9±2.3 min; there is no significant difference ($P > .05$). In similarly treated cats poisoned with tabun, the times to reappearance of breathing were: without PAM, 111±21 min; with PAM, 23±2.3 min ($.01 < P < .05$). All the animals were under artificial respiration from apnea until spontaneous respiration returned.

1221. NEW SERIES OF ANTISPASMODIC COMPOUNDS WITH SPECIAL REFERENCE TO STRUCTURE AND PHARMACOLOGIC ACTIVITY. Harold H. Bryant,* Lloyd C. Felton* and John C. Krantz, Jr. Dept. of Pharmacology, Univ. of Maryland School of Medicine, and Hynson, Westcott and Dunning, Inc., Baltimore, Md.

The pharmacologic and toxicologic characteristics of a new series of amino ethers have been studied. Special attention was given to chemical structure as related to pharmacologic activity, rate and extent of absorption and duration of effect following oral administration. The acute toxicity in mice and rats was determined both orally and i.p. and found to be relatively low. Chronic toxicity was studied on 3 members of the series in 3 species—rats, mice and guinea pigs. All 3 species tolerated 10 times the estimated maximum daily intake in man, over a period of 6 months, without evidence of toxic effects, either grossly or histologically. The pharmacologic studies of these compounds upon smooth muscle of the gastrointestinal

tract indicate a marked antispasmodic activity, either with excised muscle strips or in situ. The passage of a standard charcoal meal in fasted rats and mice was markedly decreased by oral administration of a number of these compounds. The stomach activity was likewise reduced as shown by significant extension of stomach emptying time. The duration of the inhibitory effects as observed upon the stomach was several times that shown by atropine sulfate. These effects were directly related both in degree and duration to the length of the alkoxy chain on position 2 of the ring. Increasing the number of bromine atoms on the ring also enhanced the antispasmodic activity. Three members of the series, representing the salient structural variants, were shown to exhibit no significant effect upon salivation or mydriasis in doses far in excess of the estimated maximal human intake. The cervical sympathetics of the cat were unaffected. The fleeting depressor response characteristically elicited in the dog was not abolished by autonomic blockade. The full response remained after injection of atropine, nicotine, Etamon and priscoline, as well as after capillary poisoning with large doses of arsenate. Trephining of the dog failed to inhibit the depressor response.

1222. EFFECTS OF ALKYLDIBENZYLAMINES ON SCHISTOSOMA MANSONI. Ernest Bueding and Noel Penedo.* Dept. of Pharmacology, Louisiana State Univ. School of Medicine, New Orleans.

The amebicidal activity of alkyldibenzylamines has been reported by McCowen et al. (Antibiotics & Chemother. 4:753, 1954). It was found that the same type of compounds had activity against Schistosoma mansoni in vitro. Incubation of the parasites with these and related compounds (synthesized by K. Gerzon of Lilly Research Laboratories) produced irreversible paralysis of the worms, preceded by marked muscular hyperactivity. These effects were antagonized by betaine and by carnitine, but not by choline, acetylcholine, other cholinergic agents or serotonin. Lengthening of the central alkyl chain up to 6 carbons progressively increased the antischistosomal action of alkyldibenzylamines. In contrast to their effects on Endamoeba histolytica, secondary amines were considerably more potent antischistosomal compounds than their tertiary amine analogs. Quaternary amines had no activity. The secondary amines of this series had considerably higher activity in vitro than diamidines and the schistosomicidal diamino-diphenoxyalkanes reported by Raison and Standen (Brit. J. Pharmacol. 10:191, 1955). Possible modes of actions of these compounds will be discussed. (Supported by research grants from the Public Health Service and from Eli Lilly.)

1223. ACETYLCHOLINE-CHOLINESTERASE RELATIONSHIPS IN LUNG TISSUE CULTIVATED IN VITRO. Alan Burkhalter,* Marion Jones* and R. M. Featherstone. Depts. of Pharmacology and Bacteriology, College of Medicine, State Univ. of Iowa, Iowa City.

Acetylcholine (ACH) has been shown earlier to prevent a drop to 40% of control level of cholinesterase (CHE) activity in 15-day chick embryo intestine cultured for 8 days in vitro in roller tubes. ACH has now been shown to have a much more striking effect on lung tissue of the same animal - a tissue which normally has a relatively low level of CHE. The time-effect relationships of this enzyme induction and the substrate-affinity characteristics of the enzymes present before and after the incubation period will be discussed.

1224. STIMULATORY EFFECT OF DRUGS ON EXCRETION OF L-ASCORBIC ACID AND NON-CONJUGATED D-GLUCURONIC ACID. J. J. Burns, Carole Evans,* Natalie Trousof* and Jane Kaplan.* Natl. Insts. of Health, Bethesda, Md. and New York Univ. Research Service, Goldwater Mem. Hosp., New York City.

No explanation has been offered why various drugs, i.e. barbiturates, Chloretone, aminopyrine, etc. accelerate the synthesis of L-ascorbic acid (King et al. J. Biol. Chem. 135:497, 1940). Previous tracer studies suggested that the initial effect of the drug may be on the synthesis of D-glucuronolactone, a precursor of L-ascorbic acid. Further evidence for this comes from finding that barbital and Chloretone produces in rats and dogs a marked increase in urinary excretion of L-ascorbic acid and D-glucuronic acid. However, in guinea pigs, which are unable to synthesize L-ascorbic acid, only an increase in D-glucuronic acid occurs. Further confirmation of this drug effect comes from finding that D-glucose-1-C^{14} is converted to urinary D-glucuronic acid and L-ascorbic acid to a much greater extent in barbital and Chloretone-treated rats than in rats not receiving drugs. The carrier dilution procedure employed in these tracer experiments is specific only for free and not conjugated D-glucuronic acid. The total amount of D-glucuronic acid and L-ascorbic acid synthesized from D-glucose is several times greater than dose of drug administered. This drug effect is not related to any known detoxification mechanism since barbital is not metabolized in vivo but is excreted unchanged in urine. Administration of barbital or Chloretone to hypophysectomized rats results in little or no increase in L-ascorbic acid excretion. These results suggest a hormonal control over the phenomenon which may explain why drugs of such widely different chemical structure effect the synthesis of L-ascorbic acid and D-glucuronic acid.

1225. SELECTIVE INHIBITION OF GLYCOLYSIS IN SLICES OF NEOPLASTIC TISSUES BY FLUOROPYRUVIC ACID. Harris Busch, Esther W. Olle and David L. Abrams (intr. by K. R. Unna). Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

An approach to the chemotherapy of cancer is selective inhibition of enzymes essential for function and growth of neoplastic tissues. One such enzyme is lactic dehydrogenase which catalyzes the major hydrogen transport mechanism of tumors, i.e., hydrogenation of pyruvic acid. The only compounds which have been found to inhibit lactic dehydrogenase selectively are oxamic acid, oxalic acid and fluoropyruvic acid (Busch. Fed. Proc. 15:229, 1956). The present study was designed to determine whether these compounds could selectively suppress glycolysis in slices of rat tissues, including Walker 256 carcinosarcoma, kidney and liver. In tumor, liver and kidney slices, respectively, concentrations of oxamic acid or potassium oxamate ranging from 0.003 to 0.013 M resulted in a suppression of lactate formate of 15%, 86% and 46% and a suppression of oxygen uptake of 12%, 18% and 0%. Similar concentrations of oxalic acid or sodium oxalate inhibited lactate formation 26%, 87% and 28% and inhibited oxygen uptake 20%, 37% and 0%, respectively, in slices of tumor, liver and kidney. At a concentration of 0.003 M, fluoropyruvic acid inhibited lactate formation 49%, 43% and 0% and inhibited oxygen uptake 12%, 14.5% and 3% in tumor, liver and kidney slices, respectively. These studies indicate that with fluoropyruvic acid it is possible to inhibit glycolysis in tumor slices without producing significant

suppression of oxidations. (Supported by grants C-2886C from the PHS and the Jane Coffin Childs Fund.)

1226. SPECTROPHOTOMETRIC ASSAY OF BARBITURATES. Milton T. Bush. Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

During studies of uv absorption methods of determining barbiturates it has been found that the application of the commonly used "optical density difference" (ODD) procedure can be considerably facilitated by taking advantage of the fact that suitably prepared boric acid-potassium hydroxide solutions of various pHs have practically the same optical density. It is thus possible to use the final alkaline solution (obtained by extracting the methylene chloride extract from tissue or urine at pH 7.4) as its own reference standard as follows: the solution of barbiturate in 9 ml of 0.50 N KOH is divided into 2 4.5-ml portions, 1 of which is brought to pH 10 by dissolving in it 200 mg of solid boric acid. The ODD of these solutions is then determined directly by putting the pH 13 solution in the "sample" curvette and the pH 10 solution in the "reference" curvette of the Beckman DK-1, and scanning from 400 to 230 mμ—the ODD curve shows a characteristic "positive" peak at 260 mμ and swings to an equally characteristic "negative" peak at 239 mμ. Extracts of "normal" blood and urine give practically straight lines over this wavelength range (Goldbaum. Anal. Chem. 24:1604, 1952). This type of measurement can be extended to include pHs down to at least 7, and thus can be applied to the method of Born as developed by Butler (J. Pharmacol. 100:220, 1950). (Supported, in part, by PHS Research Grant RG 3615.)

1227. CAROTID CHEMORECEPTOR STIMULATION BY NICOTINE AND SOME SYMPATHOMIMETIC AMINES. Robert Byck (intr. by J. H. Comroe, Jr.). Dept. of Physiology and Pharmacology, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

A quantitative method for measuring carotid chemoreceptor respiratory stimulation has permitted re-evaluation of the "nicotine-like" activity of some sympathomimetic amines described by Barger and Dale. Compounds tested were phenethylamine, tyramine, their methyl, dimethyl and trimethyl derivatives, and the trimethyl derivatives of 3-OH tyramine and norepinephrine. Graded intracarotid doses of these drugs and nicotine were administered to dogs anesthetized with morphine sulphate and chloralose. Respiration was measured by means of a modified Donald-Christie apparatus. The percentage increase in tidal volume of the single breath response above the median volume of the 9 preceding breaths was plotted against logarithm of dose; 30% response levels (ED_{30}) were interpolated from the straight line of best fit. Intracarotid injection of each of the above compounds produced respiratory stimulation, abolished by section of Hering's nerve. The relative order of potency of these drugs corresponds with their "nicotine-like" activity as assayed on the isolated rabbit jejunum (Pelikan 1956). Reflex respiratory response to other sympathomimetic amines as well as the mechanism of chemoreceptor stimulation are under investigation.

1228. EFFECT OF DIURETICS ON RATE OF PENETRATION OF TRICHLORACETIC ACID INTO RAT KIDNEY AND LIVER TISSUE. E. J. Cafruny (intr. by M. H. Seevers). Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Excised tissues placed in a 10% solution of trichloracetic acid are rapidly bleached. A distinct wave front is visible while the acid solution is penetrating and the penetration distance can be easily measured. On the assumptions that differences in density of tissues will result in a modification in the distance a fluid will penetrate per unit time, and that diuretics will increase kidney volume, studies were initiated in which penetration rates in tissues of control animals and animals treated with diuretic compounds were compared. Thiomerin significantly increased the rate of penetration of trichloracetic acid into kidney tissue but had no effect on penetration into liver tissue. Neohydrin, Salyrgan, Diamox and Pitressin did not alter penetration rates either in liver or kidney. Cysteine was also without effect. Examination of kidneys of rats treated with Thiomerin (5-20 mg/Hg/kg) revealed marked paleness indicating cortical ischemia. In contrast only a slight amount of or no discernible ischemia was observed in kidneys of animals treated with Salyrgan (20 mg/Hg/kg) and Neohydrin (20-40 mg Hg/kg). It is suggested that Thiomerin may produce a diversion of blood flow from cortical to subcortical levels and that this may be related to the increase in penetration rates observed.

1229. ANALGETIC EFFECTIVENESS OF SKF 5137 IN MAN. B. Calesnick. Dept. of Clin. Pharmacology, Hahnemann Med. College, Philadelphia, Pa.

Investigations were undertaken to study the clinical effectiveness of SKF 5137 as an analgetic agent. This compound is dl-N- (2:2-diphenyl-3-methyl-4-(N-morpholino)-butyrl)-pyrrolidine HCl and is a congener of methadone. It was found that the TD_{50} was 5.65 mg/patient or approximately 0.9 mg/kg. The end point was any of the following untoward reactions: drowsiness, lightheadedness, vertigo or nausea. In a group of 14 adult subjects with acute pain, SKF 5137 afforded complete relief in 1/2-1 hr and the duration was 2-5 hr. The drug was administered orally in a dosage of 5-10 mg. One case of marked sedation was reported. Another group of subjects with chronic pain is under study to determine any complications of long range therapy. There is evidence of tolerance although psychic disturbances and dependence have not been demonstrated as yet. Clinically, the analgetic potency orally appears to be greater than meperidine. In addition, 35 patients with acute visceral pain were injected subcutaneously with this drug in dosages of 3-10 mg. The results were disappointing. This finding may be explained by in vitro studies which were performed. It was found that only a slight shift in the pH of the acid salt precipitated the base. The base. The potential use of SKF 5137 would be in the oral relief of chronic pain associated with neoplastic diseases.

1230. ACUTE TOXIC EFFECTS OF CORTISONE ON THE HEART. Carolyn Cannon* and Walter M. Booker. Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.

Previous studies in our laboratory (1955-1956) have demonstrated that cortisone and some of its congeners when administered to isolated perfused rabbit heart preparations or to intact dogs cause disturbances of heart action. Rate, rhythm and stroke are greatly altered in the isolated rabbit heart preparation and in the intact dog there are similar disturbances and evidence of diminished conduction as monitored by the electrocardiograph machine. We regarded it important to determine limits of tolerance of dogs to i.v. administered cortisone; and to observe the pattern of toxicity and subsequent death following administration of the drug. Dogs are first anesthetized with pentobarbital sodium (30 mg/kg) and control electrocardiograms

are taken. Also, blood samples are drawn for determination of sodium and potassium. Cortisone is administered i.v. every 5 min beginning at 3 mg/kg and going arithmetically upwards until clear-cut signs of greatly disturbed heart action appears. We have found that when successive doses reached 9-12 mg/kg cardiac action was greatly embarrassed and usually death occurred. The electrocardiographic picture was similar to that of digitalis intoxication with markedly depressed S - T segment and slurred QRS complex. Administration of potassium early when this picture appears tends toward restoration of the normal ECG pattern, although we have not yet been able to reverse the cardiac effects of cortisone.

1231. HYPOCALORICALLY FED RETARDED RATS AS TEST SUBJECTS IN THE STUDY OF DELAYED EFFECTS OF X-IRRADIATION. H. W. Carroll* and R. W. Brauer. U.S. Naval Radiology Defense Lab., San Francisco, Calif.

Rats maintained from weaning on hypocaloric diets (about 20% of caloric intake of rats fed ad lib) which virtually arrest growth have been reported to "age" more slowly than freely fed controls. A series of such animals were subjected to repetitive X-irradiation. Hypocalorically-maintained rats were found to be considerably more radiosensitive than their normally fed controls. Other differences noted were the absence in the hypocaloric group of the anorexia and weight loss which is observed in normally fed controls. A 2nd series of these hypocalorically fed "age retarded rats" were then irradiated with 800 r (200 r/wk) 250 kvp X-irradiation, which in test runs had failed to kill any of the restricted rats. Restriction of food intake was continued for 45 days following exposure. At this time the irradiated rats and their nonirradiated controls were realimented in such a manner as to provide an increased food intake of 1 gm/day/wk/rat. The increase was continued until the rats failed to eat all the food offered them in 1 day. Results of body gain in these animals indicate that the previously irradiated rat fails to respond to the realimentation schedule in the same fashion as their nonirradiated controls. Data describing the nature of the differences observed in the irradiated and nonirradiated realimented restricted rats in terms of such factors as tissue disproportion, gross body composition, etc. will be discussed.

1233. COMPARISON OF THE ANALGESIC POTENCY AND SIDE EFFECTS OF ANILERIDINE AND DEMEROL IN MAN. Francis F. C. Chang,* Peter Safar* and Louis Lasagna. Dept. of Anesthesiology, Baltimore City Hosps. and Dept. of Medicine, Johns Hopkins Med. School, Baltimore, Md.

Thirty patients were anesthetized with nitrous oxide prior to minor gynecologic surgery under comparable experimental conditions. Anesthesia was supplemented by i.v. anileridine (N-[B-(p-aminophenyl) ethyl] 4-phenyl-4-carboethoxypiperidine HCl) or Demerol (meperidine), the choice of drug being randomized and medications given "blind." Analgesic potency was measured by the amounts of drug required to "reinforce" nitrous oxide analgesia. Anileridine, 50 mg, appeared approximately as potent as meperidine, 100 mg. Respiratory depression following doses of Anileridine required to maintain adequate analgesia was greater than that seen with meperidine. The incidence of postanesthetic nausea and vomiting was similar with both drugs. The respiratory response to 5% CO_2 and the circulatory response to sudden 70° head-up tilt were compared under double blind controlled conditions before and after i.v. injection of Anileridine, 60 mg, or meperidine, 100 mg, in 7 healthy, conscious volunteers. The response to CO_2 was depressed for longer than 2-1/2 hr after each drug in 6 of the 7 subjects. While breathing room air, the lowest respiratory minute volumes occurred between the 1st and 10th min after injection. This depression of respiration was greater after Anileridine than after meperidine. Tilt failed to cause fainting or significant arterial hypotension. Late nausea and vomiting (while ambulatory) occurred in 6 of the 7 volunteers after Anileridine and in 1 of the 7 after meperidine. After Anileridine, 30 mg, (studied in 5 of the 7 subjects), maximal depression was equal to or greater than that produced by meperidine, 100 mg.

1234. ANTAGONISM OF NICOTINE AND SOME CNS DEPRESSANTS IN MICE. Graham Chen and Barbara Bohner.* Research Labs., Parke, Davis and Co., Detroit, Mich.

Rapid i.v. injection of 0.1 ml/10 gm of a 2% solution of nicotine tartrate in mice results immediately in clonic seizures followed 5-7 sec later by maximal tonic-extensor seizures of the limbs and death. The tonic-extensor component was found suppressed first before the clonic seizures by minimal effective doses of the following compounds: morphine, isonipecaine, methadone, meprobamate, barbital, atropine, hyoscine, benactyzine, diphenhydramine, phenergan and hexamethonium. The order of the antiseizure effect was reversed with chlorpromazine. On the other hand, in pentylenetetrazol-induced convulsions, chlorpromazine was, like the other compounds mentioned above, more effective in suppressing the tonic-extensor phase than the clonic phase of the seizures. Whether a difference in the sites of action or in the intensity of stimulation by nicotine and pentylenetetrazol may account for the difference in the order of anticonvulsant effect of chlorpromazine on seizure patterns induced by the 2 convulsive agents, remains to be investigated.

1235. ACUTE HEMORRHAGIC EFFECTS OF A MIXTURE OF CELLULOSE GUM AND VEEGUM ADMINISTERED INTRAPERITONEALLY. James Y. P. Chen. Dept. of Pharmacology, Marquette Univ. Med. School, Milwaukee, Wis.

Unusual bulging and bleeding of the eyeballs and swelling of the head were produced in about 40% of mice within 4 hr following i.p. injection of 0.3 ml/20 gm of a mixture of 2.5% of CMC LV (sodium carboxymethyl cellulose, cellulose gum) and 0.5% of Veegum HV (a colloidal aluminum magnesium silicate), both of which have been extensively used as suspending agents in oral pharmaceuticals. The bulging eyeballs rapidly increased in size and usually ruptured in 6 hr. The acute swelling of the head subsided within 24 hr. Histopathological studies revealed retinal detachment, choroidal congestion, subconjunctival hemorrhage and subcutaneous edema of the scalp. No significant changes were observed in abdominal viscera. The ruptured eyeballs of those which survived the hemorrhagic episode would slough off in a few days. Rats receiving 1 ml/100 gm of such mixture i.p. showed bloody urine within 4-5 hr without changes in the eye and almost all survived the acute hemorrhagic reactions. Gross and histopathological examinations disclosed hemorrhages in the gastrointestinal tract, kidneys and urinary bladder, some breakdown of renal tubules and evidence of prominent phagocytic activity in the liver. The injected animals occasionally also developed marked and irreversible engorgement of the tongue. Pretreatment with high doses of cortisone, antihistaminics, vitamin K, ascorbic acid or

hesperidin did not inhibit the hemorrhagic reactions. However, oral administration of the mixture or intraperitoneal injection of CMC or Veegum alone, even in much higher doses, failed to induce such hemorrhagic effects either in mice or rats.

1236. EFFECT OF M5-11496 ON EXPERIMENTAL POLIOMYELITIS. Kenneth W. Cochran and Mary H. Jones.* Dept. of Epidemiology and Virus Lab., Univ. of Michigan, Ann Arbor.

M5-11496 is an antibiotic derived from a species of actinomycete, which has been reported by Powell to have activity against MM virus in mice, and by Asheshov and co-workers to be effective against bacteriophage. Since this substance appears to differ from other antiviral antibiotics, such as M5-8450 and helenine, in its relatively greater purity and in its toxicity for the host, it was of interest to determine its action against poliomyelitis in mice and monkeys. Young Swiss albino mice were inoculated i.p. with the MEF strain of type II poliomyelitis virus. Aqueous solutions of M5-11496 were injected by the i.p. route except on the day of virus inoculation when they were given s.c. When mice were treated with 2.5 mg of M5-11496/kg/day for 4 days beginning the day before virus inoculation, the morbidity due to poliomyelitis was 50 and 55% in 2 treated groups compared to 70 and 95% in the respective control groups. Some protection was obtained even when treatment was limited to a single intraperitoneal dose of 1.5 mg/kg given the day before virus inoculation. M5-11496 administered at the rate of 1 mg/kg/day for 4 days or 0.5 mg/kg/day for 8 days did not prevent paralysis in cynomolgus monkeys inoculated s.c. with the Mahoney strain of type I poliomyelitis virus. Although the toxicity of M5-11496 and its lack of effectiveness in monkeys make it an unlikely candidate for clinical application, elucidation of its mechanism of action may provide valuable information.

1237. EVALUATION OF THE INTERACTION OF THE GUANIDINIUM ION WITH SEVERAL NEUROMUSCULAR BLOCKING AGENTS ON A MAMMALIAN NEUROMUSCULAR PREPARATION. G. A. Condouris and A. Ghazal (intr. by W. F. Riker). Dept. of Pharmacology, Cornell Univ. Med. College, New York City.

The behavior of the guanidinium ion on the paralysis produced by d-tubocurarine, gallamine and decamethonium was analyzed utilizing the in vitro phrenic nerve-diaphragm system from rats and kittens. Guanidinium chloride exhibits a differential effectiveness against the paralysis produced by the 3 blocking agents, being most effective against gallamine, less effective against d-tubocurarine and variably effective against decamethonium. In both rat and kitten preparations, concentrations of guanidinium chloride from 0.1 to 0.5 mg/ml reversed all degrees of gallamine paralysis. With similar concentrations, the guanidinium ion was ineffective in restoring the twitch in muscles completely paralyzed by d-tubocurarine but was effective in increasing the twitch height in partially paralyzed muscles. Out of 14 preparations in both animal species, the contractile height reduced by decamethonium was increased by guanidinium chloride in a limited number (25%) of experiments. No effect was observed on completely paralyzed muscles and there was no definite indication of summation with decamethonium. When guanidinium chloride exhibited an attenuation of paralysis with any agent, the onset occurred within 1 min and the maximum effect in 3-5 min. When the guanidinium ion was given alone in concentrations similar to those in the above experiments, the maximal twitch was augmented. However, the onset for this action was delayed for about 5-15 min and the maximum potentiation of the twitch occurred in 15 to over 30 min. It appears, therefore, that the antiparalytic action of guanidinium chloride is not related to its potentiating effect.

1238. EFFECTS OF DICHLORPHENAMIDE ON RENAL HEMODYNAMICS AND ELECTROLYTE EXCRETION. A. P. Crosley, Jr. and R. E. Cullen (intr. by C. W. Crumpton). Cardiovascular Research Lab. and Dept. of Medicine, Univ. of Wisconsin School of Medicine, Madison.

Dichlorphenamide was administered i.v. in a dosage of 200 mg to 8 patients with cardiac and/or renal disease. Within 15 min following administration the rate of water excretion and urinary pH increased. The latter was due to increases in the excretion rates of sodium and potassium which were out of proportion to an increased rate of chloride excretion. These changes occurred despite proportionate decreases in glomerula filtration rate, electrolyte loads and effective renal plasma and blood flow. Although PAH extraction fell it was not sufficient to account for the diminution of effective renal blood flow since total renal blood flow, as measured by right renal vein catheterization, also declined. Furthermore renal blood flow/100 gm of tissue as measured by the nitrous oxide technique, also fell. Such effects persisted throughout the 60 min of study.

1239. ELECTROLYTE STUDIES ON CORONARY DOG PREPARATIONS. John R. Cummings* and Byron B. Clark. Dept. of Pharmacology, Tufts Univ. School of Medicine, Boston, Mass.

The concentrations of K, Na, Ca and Mg in coronary venous and arterial plasma were determined for an 80 to 11-hr period following 2-stage ligation of the anterior descending coronary artery. At the completion of the experiment, the electrolyte content of infarcted and noninfarcted cardiac muscle was also determined. Control determinations were made on sham-operated animals. Plasma K, Na and Ca were determined by flame photometry and plasma Mg by a spectrophotometric method developed in this laboratory (Levine and Cummings. J. Biol. Chem. 221:735, 1956). Cardiac tissue was first ashed; K and Na content was then determined by flame photometry, and Ca and Mg content by using Eriochrome Black T and appropriate piperidine-HCl buffers. At the time the ventricular ectopic rhythm developed, 8-10 hr postligation, plasma levels of K and Mg were increased and those of Ca and Na were decreased. Changes in arterial and coronary sinus blood paralleled one another closely. Statistical analysis of paired data on plasma electrolyte concentrations from the coronary dog and the control experiments will be presented. The tissue studies revealed that the infarcted tissue, as compared with noninfarcted tissue, contained 48% less K, 30% less Mg, 71% more Na and 10% more Ca. In the control experiments there were no appreciable electrolyte differences between the samples of tissue supplied by the left anterior descending artery and other samples of ventricular tissue.

1240. TACHYPHYLAXIS TO PHENYLPROPANOLAMINE AND METHOXAMINE IN PERFUSED VASCULAR TISSUE FROM DOGS. Ernest A. Daigneault (intr. by Robert A. Woodbury). Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.

The heart and the hind leg of the dog were isolated and perfused with Ringer-Locke's solution in 20

experiments and with dog blood diluted 1:1 with Ringer-Locke's solution in 20 additional experiments. Similar perfusion studies were conducted on the kidney of 20 animals, substituting Tyrode's solution for Ringer-Locke's solution. The intra-arterial non pulsatile flow was recorded with a suitable Wilson Rotameter to demonstrate the effects of 10 repeated doses of phenylpropanolamine or methoxamine. The 10th dose of each drug was increased to guarantee an adequate challenging dose. The criterion of tachyphylaxis was a lack of response even when the large challenging dose was administered. The initial injection of phenylpropanolamine markedly reduced flow. Tachyphylaxis to phenylpropanolamine was observed only in hind legs perfused with diluted blood. Methoxamine had no significant influence on coronary flow, but did reduce flow to the perfused kidney and hind leg. Tachyphylaxis did not develop to methoxamine. The fact that tachyphylaxis to phenylpropanolamine was produced in the isolated leg where blood flow returned to approximately control values after each injection is critical proof that central reflex mechanisms are not involved in either the development of this tachyphylaxis or the return to control flow values. Apparently some local mechanism is involved. The fact that tachyphylaxis did not develop in the perfused hind leg when Ringer-Locke's solution was used, but did develop when blood was the perfusate is evidence that blood furnishes one or more substances or conditions required to develop tachyphylaxis.

1241. MEMBRANE POTENTIAL CHANGES DURING ACTIVATION OF SMOOTH MUSCLE. Edwin E. Daniel (intr. by James G. Foulks). Dept. of Pharmacology, Univ. of British Columbia, Vancouver, Canada.

Previous studies in this laboratory indicated that smooth muscle exposed to sodium-free solutions became inexcitable after a prolonged contracture and that epinephrine excitation was accompanied by increased potassium outflow, but a decreased sodium uptake. These observations were contradictory in that the 1st suggested and the 2nd denied the necessity of an inward sodium current during excitation. To elucidate these problems a study of the membrane potentials changes on excitation of smooth muscle was carried out with microelectrodes. Numerous technical problems beset such studies, especially the occurrence of very high and variable input resistances (100-3000 Mohms) depending on the microelectrode and the tissue. It was possible to show that activation of cat myometrium and rodent taenia coli was accompanied by repetitive action potentials (about 20-30 mv in cells with initial potentials of 50 mv). No correlation between tension and membrane potential could be demonstrated when changes in input resistance were eliminated. Activation was not dependent on repetitive active potentials, since at 24°, it occurred in the absence of such potentials. At such temperatures, it has not so far been possible to find consistent membrane potential changes accompanying activation. These findings will be discussed in relation to experimental limitations and possible implications as to mechanisms of smooth muscle activation. (Supported by a grant from the Natl. Research Council of Canada.)

1242. EFFECT OF CHLORPROMAZINE ON ANTIDIURESIS PRODUCED BY 5-HYDROXYTRYPTAMINE IN RATS. S. R. Dasgupta (intr. by Eva King Killam). School of Tropical Medicine, Calcutta, India.

Since it has been shown that chlorpromazine fails to counteract the effect of the centrally liberated ADH, but nothing is known about its effect on the stable antidiuretic substance of the serum (5-HT), studies have been undertaken to investigate the influence of chlorpromazine on the antidiuretic effect of 5-HT. Male white rats (6 groups of 5 each) were hydrated (5 cc/100 gm) and urinary output measured at scheduled hours until 80-100% of the load was excreted (in 240 min). Each group was similarly hydrated and treated in successive experiments with: 1) 5 mg/kg of chlorpromazine, given i.p. 30 min before hydration; 2) 40 μg/100 gm of 5-HT, given s.c. immediately following hydration; 3) 5 mg/kg of chlorpromazine, 30 min before and 40 μg/100 gm 5-HT immediately after hydration. It was evident from these that 5-HT alone significantly decreased the urine volume, whereas pretreatment with chlorpromazine significantly counteracted the antidiuretic action of 5-HT. Antidiuresis produced by using posterior pituitary extract (0.006 units/100 gm) in place of 5-HT was not, however, counteracted by chlorpromazine. From various reports it appears possible that 5-HT may produce antidiuresis either by a preferential constriction of the afferent glomerular arteries or by acting as a water reabsorbing hormone. The antagonistic action of chlorpromazine may well be explained by its vasodilator effect, but the possibility that chlorpromazine affects water reabsorption mechanisms may prove useful in explaining the rationale of its therapeutic use in conditions like eclampsia.

1243. DEVELOPMENT OF TACHYPHYLAXIS BY THE PAPILLARY MUSCLE TO D-AMPHETAMINE SULFATE. Floyd Davis,* J. T. Oliver* and Robert A. Woodbury. Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.

The isolated papillary muscles of dogs were used in these experiments. Muscular contractions were recorded by means of a mechano-electronic transducer. The initial addition of d-amphetamine sulfate to the bath (concentration 5 μg/ml) caused a positive inotropic response. When this dose was repeated after the papillary muscle contraction had returned to control levels, no positive inotropic effect was elicited. This was true irrespective of whether washing was or was not performed. The lack of response on the part of the muscle lasted upwards to 8 hr. Despite the unresponsiveness of the preparation to d-amphetamine sulfate a positive inotropic action was obtained with subsequent doses of l-epinephrine bitartrate, l-norepinephrine bitartrate and d-p-hydroxyamphetamine. Determinations of d-amphetamine uptake by the papillary muscle will be reported. (Supported by American Neart Assoc.)

1244. NEW FOOD CONCENTRATE AS ADJUVANT THERAPY IN DISEASE. Oscar F. Davis, Albert Levine,* Charles Meyer,* Irving Forman* and Morris Finkel.* Dept. of Pharmacology, Stritch School of Medicine, Loyola Univ., Chicago, Ill.

Clinical-pharmacologic investigations were undertaken to study a new food concentrate (Bevamino) developed to provide controlled dietary management in disease. This palatable food contains 42% protein, little fat (2%), sodium (0.26%) and calories; is fortified with every physiologic mineral and vitamin and can be employed as the total diet. Two years of preliminary observations utilizing only this preparation plus water or milk (ranging from 5 wk to 1 yr of administrations) proved it to be a safe therapeutic tool, conducive to tissue life, growth and repair. For the present investigation 202 adult patients, of both sexes, were evaluated. These patients were derived from the following clinical categories: obesity—67;

hypertension—41; gastrointestinal diseases (regional ileitis, ulcerative colitis)—8; congestive heart failure and edema states—13; postsurgical—15; neoplasia—8; geriatrics—20; normal—30. Results were interpreted from a selected survey using 14 accepted clinical-biochemical tests. One property manifest with this preparation is the early mobilization of water, sodium and lipid. This is reflected in the striking diminution of digitalis and hypotensive drug requirements in cardiovascular patients. In obesity weight loss was profound, yet physiologic. In the enteric diseases studied, the product was an effective source of food, well tolerated by these sensitive G-I tracts. In all groups studied, overall patient acceptance, combined with objective criteria of betterment, recommended this preparation for further research in the ancillary management of disease.

1245. EFFECT OF RESERPINE (SERPASIL) ON URINE FLOW AND ELECTROLYTE EXCRETION IN "NORMAL" SUBJECTS. Eugene A. De Felice, John Ferrante and Fredrik Berglund (intr. by A. J. Plummer). Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.

Twenty-five experiments were performed in 5 "normal" subjects to determine the effects of reserpine on urine flow and C_{Cr} (endogenous creatinine), C_{Na}, C_K, C_{Cl} and C_{OSM} during water diuresis. Reserpine, 0.5 mg, was given orally twice daily, at 7 A.M. and 7 P.M., for 6 days. Experiments were done on each subject before, as well as on, the 4th and 6th days of therapy. Results were as follows: there was no change of the diuretic response to water-loading and no change in C_{Cr}. Reserpine caused a 50-85% increase of C_{Na}, C_K, C_{Cl} and C_{OSM}. The effects were identical on the 4th and 6th days of treatment. As C_{Cr} and plasma concentrations of sodium, potassium and chloride remained constant throughout the experiments, the effect of reserpine medication was an increased urinary excretion of these ions due to diminished net tubular reabsorption. (Supported by a grant from Ciba Pharmaceutical Products, Inc., Summit, N. J.)

1246. TOXICITY OF CROTALUS ADAMANTEUS (RATTLESNAKE) VENOM AND THE ANTIDOTAL EFFECTS OF HYDROCORTISONE. William B. Deichmann, John E. Dees,* M. L. Keplinger,* John J. Farrell* and William E. MacDonald, Jr.* Dept. of Pharmacology and Surgery, Univ. of Miami School of Medicine, Coral Gables, Fla.

The acute toxicity of Crotalus adamanteus (rattlesnake) venom varies considerably from species to species. The approximate lethal doses by i.m. administration are as follows: dog, 0.64 mg/kg; rabbit, 0.94 mg/kg; guinea pig, 3.2 mg/kg; mouse, 10.7 mg/kg; rat, 25 mg/kg. The symptoms produced may be classified as local and systemic. The local effects at or near the site of injection include marked edema and pain followed by rupture of the skin, digestion of skeletal muscles and necrosis. Systemic effects include lethargy and depression, salivation, bloody diarrhea and coma leading to death. Hydrocortisone hemisuccinate sodium (Solu-Cortef) when injected i.v. into dogs 2 and 4 hr after the injection of the venom was capable of raising the number of surviving dogs from 17% in the untreated controls to 65 and 75%, respectively. Without the benefit of hydrocortisone, dogs died in approximately 18 hr. Those that succumbed with hydrocortisone survived for about 3 days. The administration of hydrocortisone did not influence the local effects which suggested that the local and systemic effects induced by this venom represent 2 distinct mechanisms. The beneficial action of hydrocortisone is not understood at present.

1247. PSYCHIC EFFECTS OF COMBINED ADMINISTRATION OF RAUWOLFIA ALKALOIDS AND IPRONIAZID. Edmund W. J. DeMaar, A. I. Miller and Harry L. Williams (intr. by C. C. Pfeiffer). Dept. of Pharmacology, Emory Univ., Ga.

Combined administration of reserpine and iproniazid has been reported to produce LSD-like symptoms in the rabbit. Careful previous trials over a 3-month period on 20 patients, institutionalized with a diagnosis of schizophrenic disorder, had shown this combination to be as well tolerated as Rauwolfia alkaloids alone and to be without effect in producing aggravation of their schizophrenic disorders. The effects of reserpine or rescinnamine in doses of 2 and 4 mg orally plus 100 mg of iproniazid have been tested in 16 healty volunteers. A double blind design and a crossover technique with administration of LSD, placebo and 2 dose levels of the drug combination were employed. The volunteer subjects had previously experienced the psychic effects of LSD in doses of 25, 50 and 100 μg. Identification of drug effects was based on written reports which the subjects prepared 8-12 hr after the drugs had been taken. Subjects proved able to identify placebo or LSD administration and the dose level of the latter. Measurements of oral temperature, heart rate, bloodpressure, pupil size and hand steadiness were made throughout the trial. None of the volunteers observed any LSD-like psychic effects while receiving reserpine or rescinnamine plus iproniazid. Only a slight drowsiness, a diffusely distributed headache and some elimination of emotional tension were reported for the drug combination.

1248. FACILITORY AND INHIBITORY EFFECTS OF CONTRALATERAL SCIATIC NERVE STIMULATION ON THE PATELLAR REFLEX. S. J. De Salva,* Y. T. Cester and N. Ercoli. Dept. of Pharmacology and Exptl. Therapeutics, Stritch School of Medicine of Loyola Univ., and Armour Labs., Chicago, Ill.

In the last 10 yr, the patellar reflex established by Sherrington and his co-workers and the accessory reflexes have been used to measure the central muscle relaxant properties of drugs. Contralateral stimulation of the sciatic nerve with high frequencies (60-120/sec) facilitates the patellar reflex, through a polysynaptic pathway as established by Lloyd. A detailed investigation of some of the parameters of stimulation (frequency, pulse duration and voltage) revealed that the effects of the contralateral sciatic nerve stimulation will vary according to the conditions selected. Forty experiments showed that in the acute spinal cat, etherized during the cord sectioning, low voltage (0.9-1.0 volt) and low frequency (1-10/sec) stimulation of the contralateral sciatic nerve will generally produce inhibition of the patellar reflex, whereas high frequencies (60-120/sec) will enhance. Similar results were obtained on the etherized decerebrated animal, but not in pentobarbitalized cats. In all types of preparations, the patellar facilitation, obtained with high frequencies (120/sec), is converted to inhibition, by increasing the potential voltage used for stimulation to 10 volts or more. Changes in pulse duration alter only the degree of responsiveness without influencing its character. According to Fuortes and Hubel (J. Physiol. 1956) the flexor motor units respond to low frequencies, the extensor ones to high frequencies. The patellar reflex inhibition obtained with low frequencies is attributed to the influence of a flexor mechanism. Correspondingly, the facilitation

following high frequencies is attributed to an extensor selectivity.

1249. DEXTRAN RETENTION IN IRRADIATED RABBITS. Lawrence E. Detrick,* Harvey C. Upham* and Thomas J. Haley. Atomic Energy Project, School of Medicine, Univ. of California, Los Angeles.

The purpose of the present investigation has been to determine tissue distribution and retention of a single i.v. injection (75 cc) of clinical Dextran in irradiated rabbits (500 r whole body) as determined by the alcoholic periodic acid-aqueous-Schiff's histological staining technique for Dextran. The shorter the postinjection time the tissues were examined the more intense the Dextran-like tissue retention in irradiated or control animals. The irradiated Dextran injected rabbit that died 8 days after infusion showed; blue to black staining granular material in the liver parenchymal and Kupffer cells: patchy deposition in the convoluted tubules, casts in the tubule lumen and an occasional loaded histiocyte in the glomerulus and in interstitial tissue. The spleen, lymphnode and bone marrow lacked retention in quantity. Lymph follicles were clear: occasional filled histiocytes were seen in the red pulp, medullary sinuses and reticular cells of the marrow. Loaded histiocytes occurred in the periphery of pneumonic lesions in the lung; about lymphoid tissue, near bronchi and within thickened alveolar walls. The islets and acini of the pancreas were negative but histiocytes in the interstitial tissue were filled. Small and large bowel showed Dextran-like retention in the histiocytes of the adventitia, in the muscle, in the lamnia propria and along the blood vessels of the villa or intestinal folds. Fine blue granules were seen in the cortical cells and interstitial phagocytes of the adrenal. Striated muscle showed little and heart muscle extensive deposition in interstitial phagocytes. The intensity of Dextran retention decreased as the postinjection time of death was prolonged and was minimal in those animals killed on the 60th day. Dextran unlike PVP does not appear to be permanently retained within cells but is slowly eliminated. (Performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Comm. and the Univ. of California at Los Angeles.)

1250. EFFECT OF STREPTOMYCIN ON AMMONIA ASSIMILATION BY PSEUDOMONAS AERUGINOSA. W. E. DeTurk. Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.

Washed cells of Pseudomonas aeruginosa assimilate the nitrogen of ammonium sulfate during the oxidation of substrates belonging to the tricarboxylic acid cycle. Assimilation is obligatorily linked with oxidation of added substrates and is inhibited by streptomycin. A study of broken cell suspensions reveals 2 reactions which may function in assimilation 1) formation of glutamate from ammonium sulfate and ketoglutarate and 2) formation of aspartate from ammonium sulfate and fumarate or malate. These reactions, which occur under anaerobic conditions and are not inhibited by streptomycin in extracts, will not take place in whole cells under similar conditions. That the substrates other than ammonium sulfate gain access to the cell, is indicated by their participation in other reactions under aneerobic conditions. Ketoglutarate will transaminate with aspartate and malate and fumarate will participate in the fumarase equilibrium. Streptomycin does not affect these reactions. These observations suggest that ammonia gains access to the cell by an active transport mechanism which requires simultaneous oxidation and that streptomycin inhibits this transport.

1251. EXCITATION OF NON-MEDULLATED VAGAL AFFERENTS BY DRUGS. W. W. Douglas and J. M. Ritchie.* Pharmacology Dept., Albert Einstein College of Medicine, New York City.

In previous experiments we have found that nonmedullated afferents in the 9th and 10th cranial nerves are associated with powerful cardiovascular reflexes (Douglas and Ritchie. J. Physiol. 134:167, 1956). The origins and receptor functions of these fibers are, however, unknown. As an approach to this problem we have examined how the nonmedullated afferents in the cervical vagus respond to certain substances known to evoke vagal reflexes. This has been possible as a result of the development of a technique which allows identification and measurement of spontaneous discharge in nonmedullated fibers (Ritchie and Douglas, Fed. Proc. this issue). It has been found that 5-hydroxytryptamine and phenyl diguanide each causes a discharge in vagal nonmedullated afferents. The effect was obtained with doses of each drug of the order of 100 μg. The greatest responses occurred when the drugs were given into 1 of the great veins, but injections into the descending thoracic aorta were also effective. It appears therefor that the nonmedullated vagal afferents excited by 5-hydroxytryptamine and phenyl diguanide originate in both the thorax and the abdomen.

1252. ROLE OF POTASSIUM IN EPINEPHRINE-INDUCED CARDIAC ARRHYTHMIAS. Peter E. Dresel and Mark Nickerson. Dept. of Pharmacology and Therapeutics, Univ. of Manitoba Faculty of Medicine, Winnipeg, Canada.

Other workers have shown that cardiac arrhythmias during timed injections of epinephrine in dogs are correlated with the steeply rising arterial plasma potassium concentration associated with potassium release from the liver. In the present experiments, rapid injection of large doses (8-20 μg/kg) of epinephrine into the femoral vein of dogs under pentobarbital anesthesia resulted in 2 periods of arrhythmia. The 1st, more easily elicited, phase of the arrhythmia occurred invariably 15-17 sec after administration of an adequate dose. It consisted of a series of 1-10 ventricular beats after which the normal pacemaker returned to at least partial control. Thirty to 40 sec later, another series of ventricular beats of up to 1 min duration occurred. This observation tended to confirm that some factor brought to the heart by the venous blood was involved in the production of the 2nd arrhythmia. Intravenous administration of potassium chloride can mimic very closely the curve of arterial plasma concentrations obtained following the injection of epinephrine. When a dose of epinephrine just insufficient in itself to produce an arrhythmia was administered together with the proper dose of potassium chloride, the plasma level of the ion rose as much as or more than it did in response to a dose of epinephrine sufficient to cause an arrhythmia. This combination failed to produce arrhythmias. Isoproterenol causes few arrhythmias and little potassium release, but has potent positive chronotropic and inotropic effects. Simultaneous administration of potassium did not potentiate the arrhythmia-inducing action of isoproterenol. It seems doubtful that the correlation between the rise in arterial plasma potassium and the advent of the arrhythmias is of causal importance.

1253. COMPARATIVE EFFECTS OF GANGLIONIC BLOCKING AGENTS ON ALIMENTARY CANAL

AND URINARY BLADDER. N. B. Dreyer. Dept. of Pharmacology, Woman's Med. College of Pennsylvania, Philadelphia.

Movements of the stomach, small and large intestines, and urinary bladder in situ, in cats were recorded under either chloralose, 100 mg/kg, or sodium amobarbital 60 mg/kg, anesthesia. The peripheral portion of the sectioned left vagus was stimulated to test synaptic transmission in the intestine. It had previously been shown that hexamethonium (C_6), 0.1-5 mg/kg i.v., raised tonus and increased motility of small and large intestines. Paralysis of intestinal movements was never observed. Urinary bladder movements were depressed but recovered sometime after administration of the drug. The recovery occurred later as the dose increased. Other ganglionic blocking agents, pentolinium and ecolid, were tested in the same manner as C_6. In the small and large intestines they produced effects similar to C_6, ecolid exerting the strongest stimulation. Tachyphylaxis occurred least with pentolinium. All caused depression of urinary bladder movements, ecolid having the longest and strongest action. The body of the stomach responded by an increase in tonus only; the pylorus behaved like the intestine. These drugs stimulated the intestine in chloralosed cats where ganglionic transmission was good. Peripheral vagal stimulation after C_6 0.1 mg/kg was unchanged, yet the drug produced the typical rise in tonus and increased motility. Under sodium amobarbital anesthesia intestinal motility was good, but vagal stimulation was ineffective, while Ach 2-5 μg/kg i.v. stimulated. Under these conditions, ganglionic blocking agents were ineffectual. With continuous i.v. infusion of the ganglionic blocking agents, the alimentary canal and urinary bladder responded in the same way as to single injections.

1254. DIETHYL ETHER ANALGESIA: DIETHYL ETHER CONCENTRATION AND SPARK IGNITION OF INSPIRED MIXTURES AND LEVEL OF DIETHYL ETHER IN ARTERIAL BLOOD. Carl M. Ebersole* and Joseph F. Artusio, Jr. New York Hosp. and Cornell Univ. Med. College, New York City.

A re-evaluation and detailed description of the first stage of diethyl ether anesthesia in man was reported by Artusio in 1954. An electroencephalographic level was delineated by Bellville and Artusio during the analgesic stage which was subjected to frequency spectrum analysis and found to contain predominantly 24-cycle activity. Fourteen unselected patients undergoing cardiac surgery were premedicated with 0.0002 gm of atropine sulphate and anesthetized with diethyl ether as previously described by Artusio. Electroencephalographic activity was monitored continuously providing an objective criterion of depth. Simultaneously, 10 cc of arterial blood were taken from the arch of the aorta. These samples were analyzed for diethyl ether concentration by the method of Ronzoni and Kibler as modified by Gilder. Samples of the inspired mixture were also collected in the Thomas spark ignition chamber in order to determine its explosibility during maintenance analgesia. The electrocardiogram, blood pressure, pulse and respiratory rate were monitored continuously during analgesia. A range of values for all samples of inspired mixture collected was 0.6 volume % to 1.6 volume % with a mean of 1.1 volume %. Arterial samples showed a range of 16 mg % to 33 mg % with a mean of 27 mg %. The air to blood ratio of at least 2 hr was 1-9. In no instance did any of the analgesia ether mixtures in the inspired air produce either a flash or an explosion.

1255. CHLORPROMAZINE BLOCKADE OF WATER IMBIBITION BY FROG GASTROCNEMIUS. Eileen T. Eckhardt* and William M. Govier. Dept. of Pharmacology, Research Div. Schering Corp., Bloomfield, N. J.

Chlorpromazine at 1.6×10^{-3} M produces marked inhibition of water uptake by intact frog muscle shaken in distilled water. This block appears unrelated to antihistaminic, adrenergic blocking, or tranquilizing properties of the drug. Selected compounds exhibiting carbonic anhydrase inhibition, anticholinergic activity, analgetic and anti-inflammatory activity, adrenergic activity, monamine oxidase inhibition, cholinesterase inhibition, metabolic uncoupling and high energy phosphate supply produce little or no effect on water uptake. Correlation exists between block of water imbibition and loss of sodium and potassium from the muscle.

1256. EFFECTS OF ESTROGEN AND RELAXIN UPON PHYSICAL CHEMICAL PROPERTIES OF ACETIC ACID-SOLUBLE COLLAGEN. Harry R. Elden, Raymond J. Sever, Nancy L. Noble and Robert J. Boucek (intr. by William B. Deichmann). Howard Hughes Med. Inst., Miami, Fla.

The effects of estrogen and relaxin (Warner-Chilcott Labs.) upon the physical chemical properties of the 0.5 M HAc-soluble collagen of biopsy-connective tissue obtained from intact male and female and oophorectomized rats were followed serially by the simultaneous implantation of 3 sponges in the dorsum of the rat. The first sponge-biopsy tissue was removed after 100 days' growth and represented pretreatment tissue. After 10 days' parenteral hormone administration, the 2nd and 3rd tissue biopsies were removed. Acid solutions of collagen were prepared by centrifugation at 90,000 g for 1 hr at 6°C. Estrogen treatment resulted in an increase in the K value of the Arrhenius viscosity equation in all animals. Relaxin caused a reduction in the K value of the male and spayed rat. Intrinsic viscosity was significantly greater in the male (7.1 ± 1.2) than in the intact (3.7 ± 1.1) or spayed female and appeared not to be altered by treatment. Molecular configurations were calculated from light scattering measurements. Pretreatment male and oophorectomized rats were coil-like and the intact females were spherical. Estrogen induced a transition from a coil to a sphere in all animals which reverted during relaxin treatment. Sedimentation coefficients and their concentration dependence were determined on the oophorectomized rats in order that the molecular weights could be calculated with the Mandelkern equation. Estrogen caused an increase in molecular weight which returned toward normal with relaxin. (Supported in part by grant-in-aid, Gustavus and Louise Pfeiffer Research Fndn.)

1257. SURVEY OF D-LYSERGIC ACID DIETHYLAMIDE (LSD) ANTAGONISTS. John T. Elder, Jr.,* John H. Gogerty* and James M. Dille. Dept. of Pharmacology, School of Medicine, Univ. of Washington, Seattle.

Compounds broadly classified as ataraxics, anticholinergics, ganglionic blocking agents and adrenergic blocking agents were tested as antagonists to d-lysergic acid diethylamide (LSD) in cats. LSD in i.v. doses of 400 mcg/kg. produces a consistent behavior pattern characterized by coordinated aggression to standardized visual and tactile stimuli. An arbitrary scale of 0-5 was used to evaluate the intensity of response and the duration of LSD activity was noted. The compounds were administered by various routes

and in varying time relationships. Sixteen millimeter motion pictures were taken to allow careful analysis. Of the ataraxics chlorpromazine, meprobamate, azacyclonol, promazine and reserpine, only chlorpromazine (5 mg/kg i.v. daily for 3 days) was consistently effective in abolishing the LSD reaction. Reserpine, 0.5 mg/kg i.m. daily for 7 days, appeared to intensify the reaction and caused development of a persistent (48 hr) catatonic-like state after the aggression subsided. A similar reaction was produced by dimenhydrinate 5 mg/kg i.v., given immediately before LSD. Atropine and scopolamine administered i.m. 1 hr prior to LSD did not alter the reaction markedly, whereas hexamethonium and atropine methyl nitrate given i.v. 5 min before LSD changed the response to one of fear and withdrawal rather than aggression. Dibenzyline, 10 mg/kg i.v. 45 min before LSD, reduced the aggressive behavior markedly in degree and duration. Since peripheral cholinergic blockade and ganglionic blockade were for the most part ineffective as LSD antagonists, it appears that possibly a peripheral or a central adrenergic mechanism is implicated in the LSD response.

1258. EFFECTS OF EPHINEPHRINE ON GLYCOGENOLYSIS, PHOSPHORYLASE, AND GLUCOSE-6-PHOSPHATE IN VARIOUS TISSUES. Sydney Ellis, Josephine McGill* and Hilton L. Anderson, Jr.,* Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Pa.

Any hypothesis which attempts to relate the pharmacological effects of epinephrine to its glycogenolytic effect or to the products of increased glycogenolysis requires, obviously, a demonstration that the glycogenolytic effect of epinephrine is indeed an effect common to various tissues pharmocologically responsive to epinephrine. Although the glycogenolytic action has been adequately established and its mechanism determined in liver and voluntary muscle, this action has not been proven to the satisfaction of all for cardiac muscle. There is only meager evidence for the glycogenolytic effect of epinephrine on other tissues. In order to obtain some of the required information we have determined the effects of epinephrine on glycogenolysis, on phosphorylase activity and on glucose-6-phosphate concentration in various rabbit tissues in vitro. In confirmation of published reports it was found that in rabbit liver slices and in segments of rabbit diaphragm epinephrine increased glycogenolysis, phosphorylase activity and the glucose-6-phosphate concentration. Epinephrine produced these changes also in cardiac slices and uterine segments. In bladder segments and cerebral cortical slices epinephrine increased glycogenolysis. In intestinal strips no changes in any of the 3 factors occurred. In kidney slices glycogenolysis was slightly increased, phosphorylase activity was increased, but the concentration of glucose-6-phosphate remained unchanged.

1259. EFFECT OF NALORPHINE ON INHIBITION OF ACTH RELEASE BY MORPHINE IN THE RAT. Susan Epstein,* Hugh Burdette* and Paul L. Munson. Biological Research Labs. and Depts. of Physiology, Prosthetic Dentistry and Pharmacology, School of Dental Medicine and Med. School, Harvard Univ., Boston, Mass.

Experiments with morphine and nalorphine were carried out on young male albino rats anesthetized with pentobarbital, using histamine (10 mg/kg) as a stressful stimulus and depletion of adrenal ascorbic acid as a measure of ACTH release. The mean adrenal ascorbic acid concentrations in mg/100 gm of adrenal 1 hr after the last injection of drug were: histamine alone, 233±12; morphine (20 gm/kg) before histamine, 394±7; nalorphine (20 mg/kg) and morphine before histamine, 215±8; nalorphine alone, 402±5; nalorphine followed by a submaximal dose (3 mg/kg) of histamine, 279±8; histamine alone at 3 mg/kg, 276±7. Since nalorphine in the dose used did not itself result in ACTH release and did not potentiate the effect of histamine, it must have acted by antagonism against morphine. From further experiments with varying dose levels of nalorphine and morphine and a single constant dose of histamine, parallel log dose-antagonism curves were obtained, with a constant dose-ratio of nalorphine 1: morphine 4, thus meeting Gaddum's requirement for a drug-antagonist pair acting at the same receptor site. All the data obtained were consistent with the hypothesis that the inhibitory effect on ACTH release is not uniquely different from other effects of morphine on the central nervous system and that a central nervous component, probably hypothalamic, is an essential part of ACTH release in acute stress.

1260. ACTIVATING EFFECT OF TRYPTAMINE, BENZYLAMINE AND HISTAMINE ON PLASMA CHOLINESTERASE. Ervin G. Erdos,* Francis F. Foldes, Nora Baart* and Sydney P. Shanor.* Dept. of Anesthesiology, Mercy Hosp., and Section on Anesthesiology, Dept. of Surgery, Univ. of Pittsburgh, Pittsburgh, Pa.

The effect of tryptamine (I), benzylamine (II) and histamine (III) on the enzymatic hydrolysis of benzoylcholine (BeCh) by concentrated human plasma cholinesterase (Cholase) was investigated in the Warburg apparatus (substrate concentration 2×10^{-3} M), and in a uv spectrophotometer (substrate concentration 5×10^{-5} M). Activation of the hydrolysis of BeCh ranging from 43 to 93% was observed with all compounds with both methods. On a molar basis I was most effective. In the Warburg experiments with higher substrate concentrations, higher activator concentrations were required to produce identical effects. Investigation of the effect of I on the hydrolysis of a series of other substrates with Km-s ranging from 10^{-3} to 10^{-6} M, including procaine and PDMI-669 (a BeCh derivative containing 3 ethyl groups on the quaternary N) revealed no activation. Other indole derivatives (5-hydroxytryptamine, tryptophan, 5-hydroxytryptophan, dimethylaminoethylindole and dimentylaminemethylindole) containing a hydroxyl group attached to the indole nucleus and/or carboxyl or methyl groups in the side chain had no marked activating effect. Tyramine and histidine related to II and III, respectively, were also ineffective.

1261. EFFECTS OF TRIMETHADIONE ON SYNAPTIC TRANSMISSION IN THE SPINAL CORD; ANTAGONISM BETWEEN TRIMETHADIONE AND PENTYLENETETRAZOL. Don W. Esplin and E. M. Curto (intr. by Louis S. Goodman). Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

The effects of trimethadione (Tridione) on various aspects of synaptic transmission in the spinal cord have been investigated by conventional electrical recording techniques. Unanesthetized spinal cats were used in all experiments. Tridione has little effect upon the transmission of isolated impulses in either monosynaptic (2N) or polysynaptic pathways. Post-tetanic potentiation in the 2N pathway is also unaffected by the drug. Depression following transmission of an impulse in the 2N pathway is dramatically increased by Tridione. By means of orthodromic and antidromic conditioning volleys, it has been determined that the effect on depression is exerted at a presynaptic site. Because of the increase in depression produced by

Tridione, transmission during repetitive stimulation is poorly sustained. This effect is strikingly apparent in both monosynaptic and polysynaptic pathways at all frequencies of stimulation. The effects described are produced by doses of Tridione in the nontoxic-anticonvulsant range for cats (100-400 mg/kg). All the effects of Tridione are completely antagonized by appropriate doses of pentylenetetrazol (Metrazol); the increase in fatigability produced by 400 mg/kg of Tridione is antagonized by about 80 mg/kg of Metrazol. Conversely, hyperexcitability produced by Metrazol is antagonized by Tridione and the 2 drugs may be titrated against one another over a very wide range. Comparison of the effects of Tridione and diphenylhydantoin on synaptic transmission provides contrasts which may underlie the marked differences in anticonvulsant actions of the 2 drugs. Such contrasts will be discussed. (Supported by grant No. B-872, Natl. Insts. of Health, PHS.)

1262. SYNERGISTIC ACTION OF ATROPINE AND ANTIHISTAMINICS. Robert A. Evans,* Bernice Dertinger* and N. Ercoli. Pharmacology Dept. Armour Labs., Chicago, Ill.

We have studied the quantitative aspects of combination treatment with atropine and synthetic antihistaminics, in an attempt to learn more about their mechanism of action. On the isolated guinea pig ileum exposed to histamine, combinations of atropine and phenyltoloxamine (2 benzylphenyl β-dimethyl amino ethyl ether, HCl) showed synergistic action numerically corresponding to a positive additive effect. Comparable results were obtained when combinations including low doses of atropine were used to protect guinea pigs against lethal intravenous histamine. With higher atropine doses there was evidence of a combined toxicity of atropine and histamine. In studies of lethal astha produced by histamine aerosol, a definite synergism with potentiation was obtained by combining an ineffective dose of atropine with a partially protective dose of phenyltoloxamine. Similar results were obtained in the combination treatment with the relatively ineffective phenyltoloxamine and with atropine against acetylcholine. Thus the combination treatment extends also the spectrum of activity against the mediators tested, which tentatively could be explained that in the experimental asthma they are involved with each other. This potentiation is considered of therapeutic interest in view of the possibility of reducing dosages of both drugs to the point that undesirable side effects can be avoided.

1263. CENTRAL AND PERIPHERAL EFFECTS OF RESERPINE AND 11-DESMETHOXYRESERPINE (HARMONYL) ON THE NERVOUS SYSTEM. G. M. Everett, J. E. P. Toman and A. H. Smith, Jr.* Dept. of Pharmacology, Abbott Lab., North Chicago, and Dept. of Physiology and Pharmacology, Chicago Med. School, Chicago, Ill.

Normal mice placed on an ice cold copper plate show prompt piloerection followed by shivering. Within 30 min after oral doses of 50-100 mg/kg of reserpine (R) or 11-desmethoxyreserpine (DMR) mice and rats at room temperature (25°C) show a transient and spontaneous pilomotor erection lasting 5-10 min. Body temperature at this time is normal. Six to 18 hr later the pilomotor reflex is not elicited by the cold plate but shivering occurs. The treated mice show piloerection to i.v. injection of epinephrine or norepinephrine, although the effective dose is increased. Normal mice show some piloerection to large doses of acetylcholine but R-and DMR-treated mice do not. Acetylcholine is more toxic in these mice. Mice and monkeys showing the catatonic depression, reduced mobility, hunched posture and eye squinting typical of R or DMR are immediately and dramatically restored to apparently normal activity with loss of symptoms by i.v. doses of 1-5 mg/kg of desoxyephedrine. They gradually return to their previous condition as the effects of desoxyephedrine wear off. Further doses of desoxyephedrine are equally effective. Mouse activity cage studies have also shown that R or DMR do not affect the stimulating action of desoxyephedrine. These observations suggest that R and DMR may cause first a transient release of sympathomimetic amines, producing spontaneous piloerection followed by the failure of synthesis or retention of these substances. In line with this hypothesis are the biochemical findings by several investigators showing a depletion of norepinephrine in the adrenal medulla, the diencephalon of the dog and the superior cervical ganglia of the rabbit (M. Vogt) following large doses of R.

1264. CATION BINDING BY CHONDROITIN SULFATE. Saul Farber* and Maxwell Schubert. Dept. of Medicine and Chemistry, New York Univ. College of Medicine, New York City.

Binding of cations by anionic polyelectrolytes in solution has been recognized in a number of instances. In the particular case of chondroitin sulfate binding of cations could be of considerable physiological consequence. In order to get a measure of the extent of binding of Na^+, K^+ and Ca^{++} to chondroitin sulfate dialysis experiments were set up with solutions of Na^+, K^+, or Ca^{++} chondroitin sulfate inside a cellophane bag and the corresponding Na^+, K^+, or Ca^{++} chloride outside. After equilibration, the Na^+, K^+, or Ca^{++} and Cl^- concentrations inside and outside the bag were measured. The validity of the Donnan relation: $Na^+ \times Cl^- = Na^+ \times Cl^-$ was assumed and the apparent deviations from this relation interpreted as binding of the cation to chondroitin sulfate. Such binding means the formation of osmotically inactive Na^+, K^+, or Ca^{++}. In the case of Na^+, over a concentration range of 5-50 μEq/ml, the amount of cation bound was found to be constant at 0.9 mEq/mperiod of chondroitin sulfate. Over the same concentration range the amount of K^+ bound was 1.1, while the amount of Ca^{++} bound was 1.4 mEq/mperiod of chondroitin sulfate. Stated otherwise it appears that chondroitin sulfate binds an amount of Na^+ or K^+ equal to about 50% of its charge while it binds an amount of Ca^{++} equal to 70% of its charge.

1265. ACETYLCHOLINE EFFECTS ON THE NICTITATING MEMBRANE. II. Gloria Comulada Feeney (intr. by Theodore Koppanyi). Pharmacology Dept., Georgetown Univ. Med. and Dental Schools, Washington, D. C.

Studies on acetylcholine-induced contractions of the nictitating membrane before and after hexamethonium (Fed. Proc. 15:421, 1956) were continued in cats, with adrenergic blockade induced by phentolamine. Control runs were made to determine the dose-effect relationship of i.v. acetylcholine (3 gamma to 2 mg/kg). Acetylcholine-induced contractions after phentolamine (3 mg/kg) compared favorably with the controls. Additional phentolamine failed, but atropine completely abolished residual acetylcholine contractions. Epinephrine was administered before and after phentolamine blockade. The nictitating membrane became unresponsive. After initial atropinization, phentolamine diminished or abolished contractions after large doses (300 gamma to 2 mg/kg) of acetylcholine. After testing responses of acutely adrenalectomized cats to small doses of acetylcholine (10 to 50 gamma/kg), they were atropinized and control dose-effect

contractions with acetylcholine (500 gamma, 1 and 2 mg/kg) determined. As in normal animals, the nictitating membrane was unresponsive to epinephrine after phentolamine; and acetylcholine-induced contractions were greatly diminished or abolished. In chronically adrenalectomized cats, after atropinization, control runs were made with large doses of acetylcholine (500 gamma, 1 and 2 mg/kg). Ganglionic blockade was induced by hexamethonium (5 mg/kg base). Acetylcholine contractions were abolished. The pressor response to epinephrine was reversed by phentolamine and the nictitating membrane was unresponsive. These experiments show that the separable cholinoceptive receptors in the nictitating membrane react even after adrenergic blockade and bilateral adrenalectomy, which is in agreement with our previous hexamethonium findings.

1266. TOXICITY STUDIES ON PENTACHLORONITROBENZENE. J. K. Finnegan, Paul S. Larson, R. Blackwell Smith, Jr., H. B. Haag and Gordon R. Hennigar.* Depts. of Pharmacology and Pathology, Med. College of Virginia, Richmond.

Pentachloronitrobenzene (PCNB, Terraclor) is a soil fungicide used for the control of many common root rotting and damping-off diseases. The acute oral LD_{50} for the rat > 12 gm/kg when administered as an aqueous suspension of a wettable powder and 1.71± 0.20 and 1.65±0.17 gm/kg for males and females, respectively, when administered as a corn oil solution. In the rabbit the corn oil solution gave an LD_{50} of approximately 0.8 gm/kg. In a 3-month feeding study on rats, growth and survival were adversely affected in both sexes at a level of 5000 ppm and in males growth was suppressed at 2500 ppm. In a similar 2-yr study a tendency toward growth suppression occurred in females at levels as low as 100 ppm. No hematologic changes were seen and histopathologic changes were limited to fine vacuolization of liver cell cytoplasm at 5000 ppm. Liver to body weight ratios determined in the 3-month study were significantly elevated at all dietary levels except in the females at the lowest level (63.5 ppm). A 1-yr feeding study in dogs at 25, 200 and 1000 ppm showed no adverse effect on growth rate or survival. The hematologic picture was normal and histopathologic changes were again confined to the liver (cell enlargement with pale-staining cytoplasm). PCNB is stored to a significant extent in the fat of rats receivint it in their diet. PCNB increases the susceptibility of rats to CCl_4 poisoning. Whereas PCNB does not cause primary irritation when applied on a patch to the skin of man, sensitization to a second application is produced in a significant number of subjects.

1267. EFFECTS OF ADRENALECTOMY ON COBALT POLYCYTHEMIA IN THE RAT. James W. Fisher (intr. by W. F. Cantrell). Dept. of Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.

The use of cobalt for the treatment of anemia has aroused considerable interest in its mechanism of action. Adrenal cortical extract injections in rats produce a hypercullular bone marrow similar to cobalt induced bone marrow hyperplasia. Fifty four male Wistar (Purdue) rats were studied in 2 separate experiments. Daily i.p. injections of cobalt chloride (2.5 mg elemental cobalt per kg body wt. or 30% i.p. LD_{50}) were given (exp. I-65 days and exp. II-60 days) to normal and adrenalectomized rats. Adrenalectomized rats treated with cobalt when compared with controls showed a decrease in blood values (erythrocytes -34%, hematocrit -3.5%, hemoglobin -5%) 20 days after adrenalectomy, which was slightly less than the decrease shown by untreated adrenalectomized animals (erythrocytes -45%, hematocrit -6%, hemoglobin -7%). Normal animals treated with cobalt showed a significant elevation in blood values (erythrocytes +32%, hematocrit +16%, hemoglobin +12%) after 20 days ($P < .01$ for all values). Other significant increases were: ratio of adrenal weight to body weight in normal animals given cobalt; erythrocyte volumes (P^{32}-tagged cells) in all cobalt-treated animals at 60 days; M.C.V. and M.C.H. values at 20 days in all adrenalectomized animals, with or without cobalt. M.C.V., M.C.H. and M.C.H.C. values for cobalt treated normal animals fell below control values throughout the entire treatment period. Evidence of development of accessory adrenal crotical tissue may explain the elevated blood values in the adrenalectomized-cobalt-treated group during the 30 to 65-day period.

1268. EFFECT OF STRESS AND DICUMAROL ON PROTHROMBIN TIME OF RATS. L. M. Fisher,* G. J. Mogenson* and L. B. Jaques. Dept. of Physiology, Univ. of Saskatchewan, Canada.

Previous studies in this laboratory (Jaques and Chubaty. Rev. hemat. 9:523, 1954) demonstrated an increase in the prothrombin time of rats with stress-agents. This observation has been extended to electroshock. Approximately half the rats showed a marked increase in prothrombin time. When rats were maintained on Dicumarol, a much greater increase in prothrombin time resulted with stress. However, it was observed that a number of the rats showed nonsusceptibility to Dicumarol, as observed by Link with rabbits. Adrenalectomized rats given electrochock shosed no change in prothrombin time. Administration of Dicumarol to adrenalectomized rats elevated the prothrombin time value above that for control rats on Dicumarol and resulted in 100% mortality. The prolonged prothrombin time after stress was corrected with normal human and rat serum and $BaCO_3$-adsorbed bovine serum. (Supported by the Defense Research Board of Canada.)

1269. SENSITIVITY OF SUCCINOXIDASE OF HEPATOMA 134 AND OF MOUSE LIVER TO MALONATE. Jerome T. Fishgold (intr. by Windsor C. Cutting). Stanford Med. School, San Francisco, Calif.

The description of a defect in the respiratory mechanisms of tumor cells (Warburg) and the experimental proof that low concentrations of enzymes are more easily inhibited (Ackerman and Potter), indicate that there may be a level of malonate that would be more inhibitory to tumor than to nontumor succinoxidase. The comparative effects of malonate on the succionxidase system of liver and of hepatoma 134 or C_3H mice have been measured by reduction. One ml of incubation mixture contains homogenate from 10 mg of tissue, 90 mEq/l. of phosphate buffer, 10 mEq/l. of bicarbonate, 6 mEq/l. of tetrazolium and 1 mM succinate. When succinate is omitted, or when other Krebs' cycle intermediaries are substituted for succinate, no tetrazolium is reduced. Several other tetrazolium dyes are not reduced by this system. A smaple experiment is shown in the table; each figure represents mean of triplicate determinations of relative dye reduction.

mM malonate	Relative formazan production by	
	Liver	Tumor
0	100	100
0.046	85	76
0.10	74	52

mM malonate	Relative formazan production by Liver	Tumor
0.21	58	24
0.46	41	12
1.0	20	0

Statistical analysis yields a t value of 10.23 for the difference between liver and hepatoma; it seems clear that succinoxidase from hepatoma is more easily inhibited than from liver.

1270. NATURE OF HYPOTENSION PRODUCED BY ESTERS OF GERMINE. W. Flacke, J. M. Benforado, W. Mosimann and C. R. Swaine (intr. by Otto Krayer). Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

The veratrum alkaloids germerine, neogermitrine, desacetylgermitetrine and germitetrine B were investigated for the nature of their hypotensive effect. Veratridine was used for comparison. These compounds produce hypotension reflexly, but the effector mechanisms involved are not well established. A dog hindlimb, in nervous connection with the trunk, was perfused at constant pressure from a donor dog. Single doses of the veratrum alkaloids, given i.v. into the recipient animal, produced a decrease of vascular tone in the perfused hindlimb. Its time course did not parallel the hypotension in the recipient dog. In other experiments, aortic flow (cardiac output minus coronary flow) when measured in the whole animal (open chest, rotameter) decreased during the hypotensive response. In order to assess the relative importance, for the hypotension, of the decrease in flow and the decrease in vascular tone in the whole animal, pressure-flow curves were constructed. Aortic flow was restricted in varying degrees for short periods of time by clamping and the minimal resulting pressures were measured. About 8 sec were available for such measurements before compensatory reflexes altered vascular tone. Pressure-flow points obtained after administration of the individual veratrum alkaloids were compared with the control curves. For comparison, pressure-flow curves were aldo determined after administration of Dibenamine, representing pressure-flow relations during complete inhibition of vasoconstrictor tone. All germine esters studied decreased vascular tone during their hypotensive action. After appropriate doses of veratridine the decrease equaled that seen after Dibenamine. The relative influence of the 2 hypotensive factors, decrease in flow and decrease in vascular tone, varied during an individual hypotensive response and differed from substance to substance.

1271. EFFECTS OF 2-PYRIDINE ALDOXIME METHIODIDE ON A NEURO-MYAL JUNCTION. Joseph H. Fleisher (intr. by Stephen Krop). Biological Assessment Div., Chem. Warfare Labs., Army Chem. Ctr., Md.

2-Pyridine aldoxime)2-PAM), while effective as a reactivator of inhibited cholinesterase (ChE) (Wilson and Ginsberg. Biochem. et biophys. acta 18:168, 1955) has also been reported as having neuro-muscular blocking actions (Holmes and Robins. Brit. J. Pharmacol. 10:490, 1955). To obtain information on the mechanism of action of 2-PAM, the response of the isolated frog rectus abdominis muscle to acetylcholine (ACh) was measured in the absence and presence of 2-PAM. The contraction produced by ACh showed significant potentiation at 10^{-4} M 2-PAM, was maximum at 5×10^{-3} M and showed a decrease at 10^{-2} M 2-PAM. Correlated measurements of ChE activity of rectus muscle homogenates in 2-PAM showed 90% and 0% activity at 10^{-4} M and 5×10^{-3} M 2-PAM, respectively. When the preparation was first incubated with 5×10^{-5} M eserine so that maximal contraction occurred upon ACh addition, then subsequent contractions in the presence of 2-PAM showed progressive lowering above 10^{-3} M with 2/3 reduction at 5×10^{-3} M 2-PAM. This inhibition was antagonized by increasing the concentration of ACh and was readily reversed by washing. These observations suggest that 2-PAM produces neuromuscular block by increasing the threshold of the neuro-myal junction to ACh.

1272. PHARMACOLOGIC STUDIES ON NEW SERIES OF CHOLINESTERASE INHIBITORS. W. J. Fleming,* R. J. Wulf,* J. P. Long and R. M. Featherstone. Dept. of Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City.

Some of the pharmacologic properties of a series of bis quaternaries have been evaluated. The compounds are α,α'-disubstituted -4,4'-biacetanilids where the α,α'-substitutions are pyridyl (I), 2-methylpyridyl (II), 3-methylpyridyl (III) and 4-methylpyridyl (IV). The i.v. LD_{50} of the compounds were determined in mice and were found to be 2.5 mg/kg, 0.7 mg/kg, 0.8 mg/kg and 3.0 mg/kg, respectively. The cause of death was respiratory paralysis. Anticholinesterase activity was investigated by standard manometric techniques, using acetyl-β-methylcholine chloride (0.02 M) as the substrate for Winthrop-Stearns bovine erythrocyte cholinesterase (AChE), 10 units/ml and butyrylcholine chloride (0.01 M) as the substrate for dog serum cholinesterase (ChE). It was found by this method that compound III has a relatively strong selectivity for inhibiting AChE. The molar I_{50} values found were: compound I, 3.8×10^{-7} with AChE and 4.5×10^{-3} with ChE; II, 3.6×10^{-7} with AChE and 7.1×10^{-5} with ChE; III, 8.9×10^{-8} with AChE and 4.0×10^{-2} with ChE; IV, 4.5×10^{-6} with AChE and 4.5×10^{-4} with ChE. Further pharmacologic studies will be reported.

1273. EFFECT OF NARCOTIC ANALGETICS AND THEIR ANTAGONISTS ON PLASMA CHOLINESTERASE. Francis F. Foldes, Nora Baart,* Ervin G. Erdős* and Sydney P. Shanor.* Dept. of Anesthesiology, Mercy Hosp., and Section on Anesthesiology, Dept. of Surgery, Univ. of Pittsburgh, Pittsburgh, Pa.

The effects of 6 narcotic analgetics, morphine (I), levorphan (III), alphaprodine (V), Ro 2-7113 [3-allylnoralphaprodine (VI)], dihydrocodeine (VII) and meperidine (VIII), and that of 2 narcotic antagonists, nalorphine (II) and levallorphan (IV), were investigated with benzoylcholine (BeCh), procaine (proc.), acetylcholine (ACh) and butyrylcholine (BuCh) substrates. Concentrated human plasma cholinesterase (Cholase) was the enzyme source. The hydrolysis rates of proc. were measured in an u.v. spectrophotometer and those of the other substrates in the Warburg apparatus. The photometer and those of the other substrates in the Warburg apparatus. The substrate concentrations were 2×10^{-2}, 2×10^{-3} and 5×10^{-5} M for ACh and BuCh, BeCh and proc., respectively. With the exception of VI the narcotic analgetics in 10^{-3} to 10^{-4} M concentrations potentiated the hydrolysis of BeCh by Cholase 42-139%. The activating effect of the antagonists II an IV and the narcotic VI, which similarly to the antagonists also contains an allyl group, was considerably less. Similar effects were obtained on the enzymatic hydrolysis of proc. The hydrolysis of ACh and BuCh was not activated. In higher concentration most compounds inhibited Cholase. The outstanding effect of the analgetics was activation and that of the

antagonists inhibition. As an inhibitor VI again behaved like the antagonists. The results indicate that compounds containing an allyl group are better inhibitors and poorer activators than the parent compounds.

1274. PHARMACOLOGICAL EVALUATION OF CERTAIN ETHERS DERIVED FROM DIPHENYLETHANOL. Bernard V. Franko* and J. K. Finnegan. Dept. of Pharmacology and Physiology, Med. College of Virginia, Richmond.

Several dialkylaminoethyl ethers derived from diphenylethanol were investigated for anticipated pharmacological activities. These compounds are related structurally to compounds of demonstrated activity in several fields and were tested for the various activities indicated by these relationships. As local anesthetics they had little effect topically with the exception of the morpholino derivative which, as the hydrochloride, produced significant local anesthesia of rapid onset and short duration. Upon infiltration the piperidino derivative, as a 1% solution of the hydrochloride, compared favorably with a 1.5% solution of procaine. No compound showed significant antihistaminic activity. In vitro experiments using the guinea pig ileum showed the quaternary salts as having spasmolytic activity against barium chloride- and acetylcholine-induced spasms but as being ineffective against histamine-induced contractions. The tertiary amines were fairly effective against histamine-induced contractions but did not antagonize the barium chloride- nor the acetylcholine-induced spasms. In the invivo experiments employing the Thiry-Vella loop in dogs, a quaternary salt of the diethyl and of the diisopropyl derivatives was effective in decreasing intestinal motility. In anesthetized dogs a quaternary diisopropyl derivative decreased intestinal tonus significantly in small doses. The hydrochloride of the dimethyl compound caused mydriasis when instilled into the rabbit eye and was very irritating. A quaternary of the piperidino and of the diisopropyl compounds compared favorably with hexamethonium chloride and with tetraethylammonium chloride in antagonizing ganglionic stimulation produced by dimethylphenylpiperazinium iodide. This effect was seen in the inhibition of both blood pressure increase and urinary bladder contractions.

1275. URINARY EXCRETION OF 5HIAA AND CORTICOIDS AFTER MORPHINE, MEPERIDINE, NALORPHINE, RESERPINE AND CHLORPROMAZINE. H. F. Fraser, Anna J. Eisenman* and J. W. Brooks.* Natl. Insts. Mental Health Addiction Research Ctr., Lexington, Ky.

Every 2 wk, in randomized order, drugs and placebo were administered and urine concurrently collected daily for 3 consecutive days. The total dosage for 3 days in milligrams (range and average) was morphine sulfate 187-240, av. 226; meperidine 1950-2335, av. 2090; nalorphine 195-225, av. 219; reserpine 4.5-45.0, av. 21; chlorpromazine 1150-1600, av. 1446. Seven men received placebo, morphine, reserpine and chlorpromazine and 6 men received meperidine and nalorphine. Reserpine and chlorpromazine were given orally, others including saline placebo, s.c. Urine samples were analyzed each 24 hr but only totals for 3 days are presented. Each patient's total excretion of 5HIAA and 17-hydroxycorticoids, expressed as milligram per gram of creatinine, during 3 days administration of placebo was used as individual base lines. Changes after each drug are expressed as percentage change from each patient's placebo values. The range and average percentage change from placebo values for 5-hydroxy-3-indoleacetic acid were: after morphine, -17 to +60, av. +9; meperidine, -20 to +34, av. +9; nalorphine, -16 to +61, av. +20; reserpine +4 to +99, av. +52. Values of 5 HIAA after chlorpromazine were consistently negative and averaged -57%. This value may be falsely low because of possible interference by metabolities of chlorpromazine. Percentage changes of 17-hydroxycorticoids were: after morphine, -44 to +98, av. +24; meperidine, -52 to +39, av. +2; nalorphine, -18 to +23, av. +5; reserpine, +1 to +63, av. +28; chlorpromazine, -24 to -73, av. -54.

1276. EFFECT OF SOME ANTINEOPLASTIC AGENTS ON SEVERAL RESPIRATORY ENZYME SYSTEMS OF NORMAL AND TUMOR TISSUES. I. M. Fraser, R. A. Harvey and M. G. Hardinge (intr. by C. M. Gruber). Dept. of Pharmacology and Exptl. Therapeutics, College of Med. Evangelists, Loma Linda, Calif.

In the course of an investigation of possible differences in the action of antineoplastic agents, the effect of furfuralacetone (FFA) and mechlorethamine (MBA) on several respiratory enzyme systems of normal and tumor tissues has been examined. A 20-80% depression of the respiration of rat heart and liver slices and of mouse Ehrlich ascites carcinoma has been observed with concentrations of FFA ranging from 0.0075 - 0.03 M. The succinoxidase system of both mouse liver and sarcoma 180 homogenates was consistently inhibited approximately 20% by 0.0075 M FFA while cytochrome oxidase activity was practically unaffected. The glycolysis of mouse liver and Ehrlich ascites carcinoma was depressed 10-15% by 0.0075 M FFA. The activity of MBA was greater, the succinoxidase system of homogenates and mitochondrial preparations from mouse liver, hepatoma, sarcoma 180 and Ehrlich ascites carcinoma being inhibited approximately 50% by a 0.001 M concentration while the cytochrome oxidase system of these same tissues was inhibited approximately 50% by a concentration of 0.01 M. Studies of the effect of MBA on glycolysis in these tissues are in progress and will be reported. The significance of the effects of these agents in relation to their observed antineoplastic action will be discussed. (Supported by Grant C-2385 from Natl. Cancer Inst. PHS.)

1277. ULTRAVIOLET MEASUREMENT OF ACETYLCHOLINESTERASE ACTIVITY. Emery M. Gal (intr. by Gordon A. Alles). VA Hosp., Sepulveda, Calif. and Dept. of Pharmacology, Univ. of California Med. Ctr., Los Angeles.

The rapid fading of color with the nitroprusside method when using aectylthiocholine as substrate for the acetylcholinesterase determination (Mc Osker and Daniel. Fed. Proc. 1956) leads to variable degree of inaccuracy. Our method, based on the ultraviolet measurement of the CO-S bond disappearance gives an accurate measure of the enzyme activity. During the course of this work, I Tabachnick (Biochim. et biophys. acta 1956) employed the same principle, using acetylthiocholine iodide as substrate. The use of iodide salt, however, appears impractical due to its E_{max} 12000 at 226-228 mμ, which coincides with that of the acetylthiocholine iodide. Furthermore errors appear through absorption of iodide by the proteins. A commercial sample of the iodide was therefore converted into its chloride on a Dowex-1 chloride resin and the latter also synthesized by reacting acetyl-S-ethylenechloride with trimethylamine. These gave identical chemical and paperchromatographic reactions. Acetylcholinechloride was hydrolyzed by mice and pigeon brain preparates, the bond disappearance was measured at 228-230 mμ. This was completely

inhibited by 10^{-6} -10^{-7} M physostigmine. It was also noted that after a continuous drop in the optical density there was a slight upward turn of the rate curve, as usually noted with other thiols and presumably due to oxidation by trace metals. The appearance of thiol groups can also be measured, but less accurately, with the spectrophotometric indophenol method. An extension of this technique to cholinacetylase determination through measuring the bond reappearance in presence of thiocholine chloride is currently being investigated.

1278. QUANTITATIVE EFFECT OF HYDROCORTISONE ON SURVIVAL TIME OF ADRENALECTOMIZED RATS ADMINISTERED A LETHAL DOSE OF E. COLI ENDOTOXIN. George H. Gass and Ernest J. Umberger (intr. by Bert J. Vos). Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

Previous workers have shown that adrenal cortical steroids are able to alleviate the fall in temperature and increase the survival time of adrenalectomized mice given a lethal dose of endotoxin. We have found that adrenalectomized rats treated with endotoxin behave similarly and that the survival time increases with graded doses of hydrocortisone. The survival time of adrenalectomized rats treated with 0.5 mg of E. coli endotoxin i.p. increases from 85 to 105 min for control animals to a maximum of about 200-220 min for treated animals depending upon experimental conditions. Significant increases in survival time are obtained with as little as 10 μg of hydrocortisone per animal. Plotting survival time in minutes against log dose of hydrocortisone results in linearity of the dosage-response curve. The effect of divided doses and time of administration of hydrocortisone as well as the weight and sex of the animal on the quantitative response will be discussed.

1279. HYPERMETABOLISM OF RATS FOLLOWING INGESTION OF 3,3',5-TRIIODOTHYRONINE IN DRINKING WATER. Chalmers L. Gemmill. Dept. of Pharmacology, Univ. of Virginia, Charlottesville.

The experiments reported in the Am. J. Physiol. 1956, in press, in which high metabolic rates in rats were observed following ingestion of 3, 3',5-triiodothyronine in drinking water have been continued and extended by measuring basal metabolism of normal rats and mice with graded doses of triiodothyronine in the drinking water, determining the mortality of this method on rats and mice and measuring water intake and heart size in rats. When 26 gamma/100 ml of drinking water were used, the metabolism of rats rose to 50 Cal/m of body surface per hour in 33 days, with 52.5 gamma to 99 Cal. and with 105 gamma to 110 Cal. In groups of 9 rats each, on 27.5 gamma, there was 1 death in 36 days; on 55 gamma, 4 deaths and on 110 gamma, 9 deaths. On 220 gamma there were 9 deaths in 18 days. The amount of water ingested increased with the dosage and time. The heart sizes of the rats on triiodothyronine measured by weight were greater than in the control group. Comparable results on metabolism and mortality were obtained in mice.

1280. ACTION OF ERGOTAMINE ON CEREBRAL VENOUS PRESSURE OF THE DOG PREVIOUSLY TREATED BY A HISTAMINE LIBERATOR. S. B. Gertner, M. Virno, A. Carpi and D. Bovet (intr. by Lester C. Mark). Istituto Superiore di Sanita, Rome, Italy.

Previous work has demonstrated that the registration of the peripheral jugular pressure (p.j.p.) in the chloralosed dog enables variations in the cerebral circulation to be followed (Bovet, Gatti and Virno. Helv. Physiol. Acta 13:C6, 1955). By this technique, it was possible to observe that under optimal experimental conditions ergotamine, in small doses, clearly exerted a vasoconstrictor action on the cerebral vessels (Bovet, Gatti and Virno. Rendic. Accad. Lincei 19:67, 1955). This effect of the alkaloid was the more pronounced the higher the initial p.j.p. In certain animals when the p.j.p. was already low the cerebral vasoconstiction caused by ergotamine was either very small or absent entirely. The action of ergotamine has now been studied following the administration of 48/80, which had previously been found to cause a very marked rise of the p.j.p. in doses of 50 μg/kg (Virno, Gertner and Bovet. J. Pharmacol. 118:63, 1956); this action was presumed due to the release of histamine. When ergotamine was administered in doses of 5-10 μg/kg, i.v. during the state of cerebral vasodilatation produced by 48/80, it caused a pronounced fall in the p.j.p. to the pre-existing normal level. No antihistaminic action or ergotamine was evident with the doses employed. The correspondence observed between the dose of ergotamine active in the animal and that effective in clinical use and the strong vasoconstrictor action of ergotamine on the cephalic circulation under conditions of vasodilatation, is in good agreement with the view which correlates the therapeutic action of the alkaloid in migranious headache with the vascular disturbance observed during the migraine attack.

1281. PRESENCE OF 5-HYDROXYTRYPTAMINE (SEROTONIN) IN PERFUSATE FROM SYMPATHETIC GANGLIA. S. B. Gertner,* M. K. Paasonen* and N. J. Giarman. Depts. of Pharmacology, Columbia Univ., New York City, and Yale Univ., New Haven, Conn.

Although homogenates of sympathetic ganglia have been shown to possess a high capacity to form 5-hydroxytryptamine (5-HT) catalytically, when compared to other structures of the nervous system, all attempts to find preformed 5-HT in excised sympathetic ganglia of mammals have been unsuccessful. In this work, we have been able to demonstrate the apparent production of 5-HT by sympathetic ganglia in situ. This has been accomplished by the use of the monoamine oxidase inhibitor, iproniazid ("Marsilid"), in the perfused superior cervical ganglion of the cat. The 5-HT was assayed on the heart of Venus mercenaria. When iproniazid in a concentration of 200 μg/ml was added to the Locke's solution used for perfusion, there was an immediate, but partial inhibition of transmission in the ganglion. No 5-HT was demonstrable in the perfusate, however, until 2-3 hr after the beginning of the iproniazid perfusion. Thereafter, progressively increasing amounts of 5-HT appeared in the perfusate. The presence of 5-HT in the perfusate was apparently unrelated to preganglionic nerve stimulation and bore no noticeable relationship to the contractions of the nictitating membrane caused by electrical stimulation. Control experiments, followed for 6 hr, revealed no 5-HT production in the absence of iproniazid. In 2 experiments when both iproniazid and the precursor of 5-HT, 5-hydroxytryptophane, were present in the perfusion fluid, 5-HT was detectable in the perfusate within 15 min and progressively increasing amounts occurred with time. Again there was no relationship between the effects elicited by electrical stimulation and the presence of 5-hydroxytryptamine in the perfusate. In all these experiments, the 5-HT found was in the order of 10-20 mμg/min.

1282. LIDOCAINE SENSITIZATION OF THE IN VITRO RAT UTERUS TO ACTION OF EPINEPHRINE. J. E. Giering* and A. P. Truant. Astra Biological Labs., Worcester, Mass.

In previous studies, lidocaine sensitization of the in vitro sphincter iris of cattle to epinephrine was demonstrated. (Truant et al. XX Int. Phys. Cong., 1956). Experiments were extended to the in vitro rat uterus. Diestrous uteri, or uteri from animals castrated for 7-14 days were used; estrous uteri were found to be unsatisfactory. The preparations were suspended in a modified and aerated Krebs' solution and activity was recorded isometrically in a conventional manner. Muscles in a high tonal state were most suitable for demonstrating a quantitative duration of relaxation with epinephrine. With suitable doses, all local anesthetics could cause an increase in uterine tone and motility. However, only lidocaine sensitized the uterus to the inhibitory response of epinephrine and this action was independent of the lidocaine effects on the tone or motility of the uterus. Lidocaine in a final concentration of 5×10^{-2} consistently sensitized the uterus to epinephrine in a concentration of 1×10^{-4}. In this instance, the duration of relaxation was 3 to 4 times that of the epinephrine control. This action occurred rapidly and was readily removed by several washes. Sensitization was not observed with cocaine or other local anesthetics (procaine, hexylcaine, tetracaine, dibucaine, etc.). The absence of cocaine sensitization is particularly noteworthy. Uterine responses to these drugs during the phases of the estrous cycle will be discussed.

1283. MODIFICATIONS OF ACTIONS OF LSD-25 BY RESERPINE. John H. Gogerty, John T. Elder and Akira Horita (intr. by J. M. Dille). Dept. of Pharmacology, School of Medicine, Univ. of Washington, Seattle.

It has been demonstrated that lysergic acid diethylamide (LSD) induces a marked hyperpyrexia in laboratory animals. This effect has been used in this laboratory to quantitate the response of rabbits to LSD. Reserpine and chlorpromazine both induce hypothermia in rabbits. Investigations in this laboratory have shown that by varying the interval between administration of reserpine and that of LSD, the hyperpyrexia due to LSD can be either "potentiated" or inhibited. The i.v. injection of 50 μg/kg LSD 2 hr after i.v. injection of 1 mg/kg reserpine results in marked potentiation of the LSD response which culminates with death of the animal. If the interval between injections is 6 hr, the LSD response is "normal" or decreased. When the interval is increased to 10 hr, there is a 40-60% reduction in the pyretogenic response to LSD. Injection of LSD 22 hr after reserpine produces a response which resembles a "normal" LSD response except that the maximum temperature is reached at approximately 2 hr following injection instead of 1 hr. Cats premedicated with reserpine, 0.5 mg/kg i.v. daily for 7 days, demonstrate a more violent rage reaction to LSD 400 μg/kg i.v. than untreated cats, but generally the rage is of shorter duration. About 1 hr after the administration of LSD, a marked catatonic-like state develops which persists for 48 hr. During this time the cats are unresponsive to auditory, visual and tactile stimuli.

1284. DETERMINATION OF DORIDEN. Leo R. Goldbaum, Melvin Williams* and Theodore Koppanyi. Dept. of Toxicology, Armed Forces Inst. of Pathology, and Dept. of Pharmacology, Georgetown Med. School, Washington, D. C.

A method for the determination of Doriden has been developed, based on its characteristic ultraviolet absorption spectrum and its gradual disappearance with hydrolysis in alcoholic KOH. Two milliliters of plasma are extracted with 25 ml of chloroform. The choloroform is run through filter paper (Whatman 41) to obtain a clear solution, shaken with 5 ml of 0.5 N NaOH, and with water layer discarded. Five milliliters of 0.5 N HCl are then added, and the mixture shaken. The solvent layer is again run through filter paper and a clear aliquot (20 ml) evaporated to dryness at room temperature with air current. The residue is redissolved in 4 ml of absolute ethanol. A 3-ml aliquot is pipetted into a tube containing 1 ml of 0.2 N KOH. The mixing time is recorded. The optical density (O.D.) of the solution is determined in a Beckman spectrophotometer against 3 ml of absolute ethanol and 1 ml of 0.2 N KOH as reference solution. Doriden, in alcoholic KOH, has a characteristic absorption maximum at 235 mu ($E^{1\%}_{1\,cm} = 880$) and a minimum at 225 mu. The O.D. at 235 mu is recorded repeatedly at 8-min intervals. The rate of hydrolysis of a Doriden standard solution is determined under identical conditions. The half-life for Doriden dissolved in 3 ml of absolute ethanol and 1 ml of 0.2 N KOH is approximately 17 min. The O.D. at 235 mu after 10 half-lives represents absorbing substances other than Doriden. This O.D. is subtracted from each of the initial readings. On semi-log paper, the corrected O.D.'s are plotted against time. The O.D. at zero time is determined by extrapolation. Using the above procedure, 50 μg of Doriden is 2 ml of plasma will have an O.D. at zero time of approximately 0.60 at 235 mu. Recoveries of added Doriden showed an error of ±5%. This method has been applied to determine plasma levels of Doriden in humans and dogs.

1285. ACCUMULATION OF ACID-SOLUBLE NUCLEOTIDE COMPOUNDS IN AN E. COLI MUTANT DEPRIVED OF AN ESSENTIAL AMINO ACID. Dora B. Goldstein,* Avram Goldstein and Beverly J. Brown.* Cancer Chemotherapy Lab., Dept. of Pharmacology, Stanford Univ., Calif.

A leucine auxotroph of E. coli (strain K-12) was grown in minimal medium with leucine in excess or in limiting amount. Control cells were harvested in the logarithmic phase of growth at about half maximal turbidity. "Fasted" cells abruptly showed their growth due to exhaustion of leucine from the medium, then increased their dry weight 10-15% during the next 60-90 min. They were then harvested at the same total dry weight as the control culture. Cells (about 350 mg) were washed cold, sonicated, suspended in cold 5% TCA, and centrifuged. After removal of TCA the acid-soluble fraction was separated into 6 U.V.-absorbing peaks on Dowex-2 formate and each peak was then subjected to paper electrophoresis at pH 2.6 and to paper partition chromatography. In the acid-soluble fraction of "starved" cells there was an increase of about 50% in U.V.-absorbing substances, chiefly of strongly anionic character. At least 3 components were unique to the "fasted" cells; these yielded ninhydrinpositive material after acid hydrolysis. Free UMP also accumulated but inasmuch as CMP did not, we discount the possibility that ribonuclease activity per se could explain our results. The identification of the substances unique to the "fasted" cells is in progress. The bearing of these findings upon the mechanism of protein synthesis will be discussed. (Supported by grants from the Natl. Microbiological Inst. and Natl. Cancer Inst., PHS.)

1286. EFFECT OF VARYING POTASSIUM ION CONCENTRATION OF RESUSCITABILITY OF THE

ISOLATED MAMMALIAN HEART. Allen W. Gomoll* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

The report of Sherrod (1955) on the resuscitation of the mammalian heart following arrest of 1-2 hr duration has initiated much interest in the ionic environment and alterations associated with this phenomenon. In the present study, isolated rabbit hearts were perfused with normal Ringer-Locke solution at 36°C. for periods of 18-30 min, then the hearts were allowed to "die" by discontinuing the perfusion solution. After complete cessation of both ventricular and auricular activity, all hearts were allowed to remain quiescent at room temperature for a period of one hour. Cessation of activity, monitored grossly and in some cases by direct unipolar myocardiography, was observed in 6-19 min. The resuscitation of the hearts was attempted after the period of arrest by restarting perfusion of the hearts with Ringer-Locke solutions which contained 100%, 50%, 25% and 10% of the physiological amount of potassium, all other ion concentrations remaining normal. Resuscitation with Ringer-Locke solution deficient in potassium was successful in all but one case; however, the rhythm and force of contraction were not comparable to those seen in hearts resuscitated with Ringer-Locke solution containing the normal compliment of potassium. Irregularities of rhythm which were noted as the calcium-potassium ratio varied were bigeminy, trigeminy, ventricular and auricular asystoles, ventricular tachycardia and frequently fibrillation. With decreasing concentrations of potassium it was observed that the time interval before the first ventricular contraction, after restarting perfusion, became correspondingly shorter. However, this potassium deficiency resulted in a more limited duration of normal activity. (Supported in part by a grant from the PHS.)

1287. UPTAKE OF FISSION PRODUCTS AND NEUTRON INDUCED RADIONUCLIDES BY THE CLAM. J. K. Gong, W. H. Shipman, H. V. Weiss and S. H. Cohn (intr. by R. W. Brauer). U.S. Naval Radiological Defense Lab., San Francisco, Calif.

A study of the uptake of 3 neutron-induced radionuclides (Zn^{65}, Fe^{59} and Co^{60}) and a 2-month-old mixture of fission products by the clam demonstrated the ability of this marine animal to rapidly concentrate significant quantities of these radionuclides in both the shell and soft tissue. The uptake of radioactive material in the soft tissue is a metabolic incorporation, while the higher uptake by the shell appears to be a surface adsorption phenomenon. The rate of incorporation by the soft tissues was constant and was highest for the rare earth group of fission products. Uptakes of Co^{60}, Zn^{65} and Fe^{59} were also constant but occurred at much lower rates Sr^{89} and $Ru^{103-106}$ were taken up only in equilibrium with the radioactive medium in the experimental interval studied. Further study with the radionuclide Co^{60} indicated that the rate of uptake varied as a power function with the level of concentration of the radionuclide in the medium. The ability of the clam to concentrate radioelements to a high degree makes it valuable as a biological indicator of radioactivity. It may be particularly useful in assessing the contamination of marine areas with low levels of radioactivity.

1288. BENEFICIAL EFFECT OF SEROTONIN AND COMPOUND F ON E. COLI ENDOTOXIN MORTALITY IN MICE. Paul Gordon and Morris A. Lipton (intr. by J. A. Wells). VA Research Hosp. and Dept. of Pharmacology, Northwestern Univ. Med. School, Chicago, Ill.

Pretreatment with serotonin in large doses has been reported to have no significant protective effect in burn, tourniquet, and tumbling shock and has not been previously studied in endotoxin shock. That small doses of serotonin significantly reduce the mortality of shock due to endotoxin is demonstrated in the studies to be presented here. Twenty gamma of serotonin afforded significant protection to adult mice of several strains when injected s.c. 30 min before, or immediately following, an i.p. injection of purified E. Coli endotoxin (Difco). Males responded less well than females. Enhancement of the therapeutic effect of serotonin was observed if 25 gamma of compound F, suspension, was injected s.c. at a separate site at the same time. Compound F in this form and dosage had no effect on mortality in itself. In a strain of mice highly sensitive to endotoxin (Swiss-Webster), serotonin (40 gamma) and compound F, suspension (25 gamma) injected s.c. immediately following a dose greater than the LD 100 of endotoxin protected 33% of the animals. In a less sensitive strain (Carworth Farms mice) 20 gamma of serotonin and 25 gamma of compound F reduced mortality by 50%.

1289. REVERSAL BY INSULIN OF IMPAIRED HISTAMINE RELEASE BY DEXTRAN IN DIABETIC RATS. Andres Goth, W. L. Nash,* M. E. Nagler* and James Holman.* Dept. of Pharmacology, Univ. of Texas Southwestern Med. School, Dallas.

It has been shown in a previous communication that the response of alloxan diabetic rats to large molecular histamine releasers such as dextran or egg albumin is markedly inhibited while their response to the smaller molecule compd. 48/80 is unimpaired. The effect of insulin on this phenomenon was examined in the present study. Forty-one alloxan diabetic rats were divided in 2 groups. Twenty-three were pretreated with regular insulin, 4 U/200 gm s.c., 1 hr prior to the i.v. injection of 600 mg/kg of dextran. Eighteen alloxan diabetic controls received no insulin prior to dextran. Mean plasma histamine following dextran, assayed on the atropinized guinea pig ileum, was 0.38 μg/ml in the insulin treated and 0.05 μg/ml in the untreated diabetic rats. The differences were statistically significant. Since skin histamine in diabetic rats was normal, these results suggest that there is in such animals a defect in the transport of large molecular histamine liberators or in the basic process of histamine release. (Supported by the Welch Fndn., Houston, Tex.)

1290. RESERPINE ON RENAL HEMODYNAMICS IN THE TRAINED UNANESTHETIZED DOG. Henry A. Gould* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

The action of reserpine on renal hemodynamics was determined in 5 trained unanesthetized dogs in which control measurements were established for glomerular filtration rate, renal plasma flow, and the rate of urine formation. Control blood pressure determinations were made by a direct puncture of the femoral artery. One and one-half hr prior to experimental clearance studies and blood pressure determinations, reserpine was administered in a dose of 0.25 mg/kg of body weight i.m. An average of 6 15-min clearance periods were run on each of 5 dogs. Reserpine caused variable effects on blood pressure, no significant effects on either glomerular filtration rate nor renal plasma flow. However, it produced a

rather marked decrease in the rate of urine formation. This apparent antidiuretic effect, which occurred in the absence of any other significant changes in renal hemodynamics, may be mediated in some way by the release of excess antidiuretic hormone from the posterior pituitary gland. (Supported in part by a research grant from the PHS.)

1291. COMBINED EFFECT OF TRANQUILIZING DRUGS AND ALCOHOL ON RATS. R. C. B. Graham,* F. C. Lu and M. G. Allmark.* Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Canada.

The sloping screen technique used for the assay of curare by Allmark and Bachinski (J. Am. Pharm. A. Scient. Ed. 38:43, 1949) was adapted to measure the effects of alcohol and tranquilizers on the performance of rats. Wistar rats were given a small dose of a tranquilizing drug that did not cause any apparent effect in these animals. After a suitable interval the treated groups and untreated control groups received, either i.p. or orally, 3 or 4 graded doses of 50% ethanol. The rats were immediately placed on the sloping screen, and a response was usually observed within 10 min. The number of rats that slid off the screen within 30 min was recorded, and the results were analyzed statistically. The following tranquilizers were tested for their effect on the action of alcohol: glutethimide, methyprylon, meprobamate, promazine, captodiamine, N-methylpiperidyl-(3)-methylphenothiazine (Pacatal), ethinamate, hydroxyzine, ethyl, trichloramate and azacyclanol. These drugs, with the exception of hydroxyzine, azacyclanol and possibly ethinamate, enhanced the effect of alcohol on the rats. and azacyclanol produced a slight but significant decrease in the effect of alcohol. In another experiment the loss of righting reflex in rats was used as an index of alcohol action, rather than the sloping screen technique. In this case promazine enhanced the effect of alcohol as long as 10 hr after administration of the latter. From the results obtained, it is likely that in most cases the combined effect of the tranquilizers and alcohol was additive, rather than synergistic.

1292. ANALGESIC POWER OF dl-PIPERIDYL METHADONE AND MORPHINE IN MAN. Joachim S. Gravenstein,* Donald P. Pederson* and Henry K. Beecher. Anesthesia Lab., Harvard Med. School at Massachusetts General Hosp., Boston.

In a double blind study in postoperative patients with severe and moderate wound pain dl-piperidyl methadone, 15, 25 and 35 mg/70 kg body weight was compared with morphine, 10 mg/70 kg body weight. Pain relief was expressed using a score system representing complete, good, poor and no relief. No dose of dl-piperidyl methadone gave more relief or was longer acting than the morphine standard. Fifteen milligrams dl-piperidyl methadone was significantly less potent than morphine, 10 mg, both in severe and in moderate pain. Twenty-five milligrams dl-piperidyl methadone was significantly weaker than the morphine standard only in severe pain; in moderate pain it was as good as morphine, 10 mg. No statistically significant differences between the morphine standard and 35 mg dl-piperidyl methadone was seen.

1293. ANTIVIRAL ACTIVITY OF BIOFLAVONOIDS. D. M. Green, S. Yoshimura* and L. S. D' Agrosa.* Research Div., Grove Labs., Inc., St. Louis, Mo.

A series of crude and purified bioflavonoid fractions from lemon, orange and grapefruit, supplied by Sunkist Growers, Ontario, California, have been evaluated for antiviral activity. Groups of mice were systemically infected with LD_{100} doses of one or two viruses: Influenza PR_8 (a mouse-adapted human virus) or Mengo encephalomyelitis (a mouse-adapted monkey virus). Bioflavonoid fractions were administered by either the intragastric or intraperitoneal route twice daily for 5 days. The mean survival time of the group, and the number of survivors 10 days after injection, were used as criteria of antiviral activity. Several fractions were found to prolong survival and to protect significant numbers of animals from death on both i.p. and oral administration. Activity appeared to vary with the type of virus and with the nature of the bioflavonoid fraction used. Antiviral activity did not appear to depend on postulated activities toward capillaries, for highly purified hesperidin gave relatively poor protection. Since treatment of virus suspensions with bioflavonoids prior to inoculation of the test animals failed to reduce subsequent lethality, antiviral activity in vivo apparently was not due to a direct virucidal action. An indirect action, possibly involving stimulation of a natural defense mechanism, is suggested.

1294. INHIBITION OF RENAL TUBULAR EXCRETION OF N^1-METHYLNICOTINAMIDE (NMN) BY TETRAALKYLAMMONIUM DERIVATIVES IN THE CHICKEN. R. E. Green and Lawrence Peters. Dept. of Pharmacology, Kansas Univ. Med. School, Kansas City.

The simultaneous renal excretion of NMN and p-aminohippurate (PAH) by hens was studied using Sperber's procedure (Ann. Roy. Agr. Coll. Sweden 15:317, 1948). Urine was collected separately from the 2 kidneys. NMN and PAH were infused simultaneously into the renal portal circulation of 1 kidney via the leg vein. Tubular transport rates of NMN and PAH were calculated as the apparent tubular excretion fraction (ATEF): (excretion/min on infused side minus excretion/min on opposite side) amount infused/min. After 4 10-min periods of urine collection a tetraalkylammonium derivative was added to the infusion and urine collection continued. Tetraethylammonium (TEA) caused a 60% inhibition of the tubular transport of NMN when the molar ratio of TEA:NMN infused was 8.5:1. The readily reversible inhibition was less marked but highly significant when the infusion ratio was 5:1 and was absent when the ratio was 2.5:1. Tetramethylammonium (TMA) failed to depress NMN transport even when the molar ratio of TMA:NMN infused was 9.6:1. Triethylmethylammonium was only slightly less active than TEA in depressing NMN transport. Trimethylethylammonium (TMEA) produced only a barely detectable depression of NMN transport when the ratio of TMEA:NMN infused was 7.5:1. The ATEF of PAH remained essentially uniform throughout all experiments. Other tetraalkylammoniums are to be studied. (Supported by Grant No. H-2377 from the Natl. Heart Inst.)

1295. ACTION OF EPINEPHRINE AND OF ADRENOCHROME ON OXIDATION OF GLYCINE IN PRESENCE AND ABSENCE OF RAT TISSUES. Margaret E. Greig and Anna J. Gibbons.* Research Div., Upjohn Co., Kalamazoo, Mich.

Epinephrine or adrenochrome in catalytic amounts stimulated the oxidation of glycine in the absence of tissue, to CO_2, NH_3 and formic acid and small amounts of glyoxylic acid. Under similar experimental conditions glyoxylic and glycollic acids were oxiddied very slowly, compared with the rate for glycine. Thus these acids did not appear to be intermediates in the oxidation of glycine. In the

presence of rat kidney, liver or brain, epinephrine or adrenochrome accelerated the rate of oxidation of glycine to glyoxylic acid. No formic acid was detected. Thus in the presence of tissue the oxidation products of epinephrine stimulated an enzymatic reaction which normally occurs at a slow rate. The rate of oxidation by kidney or other amino acids, eg. glutamic acid, phenylalanine and tryptophane, was also stimulated by adrenochrome but the magnitude of the effect was very much less than when glycine was the substrate. It has been postulated by certain workers that epinephrine may be oxidized to adrenochrome and other oxidation products and finally to melanin in abnormally high amounts in schizophrenia. If this be so, the reaction described above, if it occur in vivo, could account for the apparent deficiency of glycine as evidenced by the decreased ability to detoxify benzoic acid (Quastel and Wales. Lancet 2:301, 1938) and the increased formation of phosphoglycollic acid (Ostrom. Arch. Biochem. 33:484, 1951) (which could be formed from glyoxylic acid) in erythrocytes reported in schizophrenic patients.

1296. THRESHOLD DOSES OF LSD IN HUMAN SUBJECTS. Theodore Greiner, Neil R. Burch* and Robert Edelberg.* Dept. of Psychiatry, Baylor Univ. College of Medicine, Houston, Tex. This work was accomplished under Contract AF 18 (603)-79 Office of Scientific Research, U.S.A.F.

In doses above 40 μg LSD produces behavioral alterations and florid perceptual distortions that have been well documented in human subjects. Performance of complex tasks is impossible and the model psychosis is obvious even to inexperienced eyes. Doses below 20 μg are of practical concern if gross performance is spared, but sporatic affect disorders yield ideational and motivational impairment; on the other hand, theoretic interest is centered about the possible similarity of the threshold "model" psychosis to incipient schizophrenia. The subjects were young, nonpsychotic males, well aware of the action of LSD. A series of doses at intervals of a week ranged from 4-20 μg, along with placebo. All data were interpreted in a double-blind state. In addition to frequent psychiatric interviews, subjects made extensive notes of their attitudes and thoughts both during and after the drug effects. An adjective check list was prepared from the earlier experiences. Bioelectric recordings included frequent samples of EEG and GSR. Pupillary-iris ratios were measured from serial photographs. Severe mood swings were outstanding with an early euphoric phase followed by depressive features. Double-blind judgment about the dosage was only fair with many inversions in the ranking. Placebo was not always distinguished, although ranked as the minimum effect. Alerting patterns in EEG and GSR were noted in the dose range above 10 μg. The dosage response relationships for specific disturbances of affect and thought will be described.

1297. EFFECTS OF "METHITURAL" ON THE CARDIO-VASCULAR SYSTEM. Charles M. Gruber and Lester H. Lonergan.* Dept. of Pharmacology, College of Med. Evangelists, Loma Linda, Calif.

Healthy mongrel dogs, under Methitural anesthesia, were used. Blood pressure changes were recorded with a mercury manometer, and cardiac changes with a Sanborn Twin-Viso Electrocardiograph. The effect of Methitural on heart rate, studied in 16 animals, was so variable no conclusions could be drawn. Fifteen injections of Methitural were made in 12 animals resulting in no change in cardiac rhythm and no change or a fall in blood pressure. When the systemic blood pressure was increased by carotid artery occlusion, stimulation of the central cut ends of either the vagus or sciatic nerve, cardiac irregularity usually occurred. Cardiac arrhythmia and a rise in blood pressure were seen after 48 injections of Methitural or in 96% of the animals. The administration of oxygen by tracheal insufflation had no influence on the cardiac irregularity unless there was also a fall in blood pressure, in which case normal rhythm resulted. Lowering the blood pressure, by hemorrhage, reverted the heart to normal rhythm and reinjection of the blood, to restore the blood pressure, caused a resumption of cardiac irregularity. Rapid injection of a small dose of Methitural in an animal under Methitural anesthesia with cardiac arrhythmia, caused a fall in blood pressure and normal heart beat. Any reduction in blood pressure, whether produced by nerve stimulation, inhalation of amyl nitrite, i.v. injection of histamine, quinidine or acetylcholine, caused the cardiac arrhythmia to disappear. Increased irritability of the heart to strain appeared to be the cause of the cardiac irregularity.

1298. RESPIRATORY AND CARDIOVASCULAR EFFECTS OF NICOTINE IN THE CAT AND DOG. Carl C. Gruhzit. Dept. of Physiology and Pharmacology, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

Relative roles and sensitivity of the various mechanisms involved in the cardiovascular and respiratory responses to nicotine were studied in chloralose-anesthetized cats and dogs by comparing the effects produced by right atrial, pulmonary arterial, left ventricular and intra-aortic injections before and after cervical vagotomy, carotid sinus nerve section, adrenalectomy, atropine and adrenergic and ganglionic blocking agents. Femoral arterial and right atrial pressures were measured by means of strain gauges and recorded on a Grass electroencephalograph. Respiration was recorded on the EEG by means of a modified Donald-Christie apparatus. The smallest effective right atrial injection of nicotine (0.25-4.0 μg/kg) produced respiratory stimulation frequently accompanied by a rise in blood pressure. The right atrial administration of 1.0-8.0 μg/kg produced, after a latent period usually less than 3 sec, an initial respiratory depression, bradycardia and hypotension followed by hyperpnea and frequently tachycardia and hypertension. Left ventricular injections of the same dose resulted in qualitatively similar effects except initial respiratory depression was not observed. The respiratory and cardiovascular depressant effects were prevented by vagotomy or the administration of sodium thiopental while the hyperpena, hypertension and tachycardia elicited by small doses of nicotine were abolished by section of both the vagi and carotid sinus nerves. After buffer nerve section, however, larger doses of nicotine still produced a rise in blood pressure which could be inhibited or abolished by adrenergic or ganglionic blocking agents. The various mechanisms involved in these responses to nicotine will be discussed.

1299. SOME EFFECTS OF ACUTE SUBLETHAL ALCOHOL INTOXICATION IN INTACT AND ADRENALECTOMIZED DOGS. Harvey B. Haag, Gerda I. Klingman* and Ruby Bane.* Dept. of Pharmacology, Med. College of Virginia, Richmond.

Acute sublethal alcohol intoxication (8 ml/kg as a 25% solution administered by stomach tube) in intact unanesthetized dogs was accompanied by the following changes: a marked hyperglycemia, a transient

hypokalemia, a slight increase in hematocrit, a decrease in erythrocyte sedimentation rate and oliguria. Anuria, lasting for approximately 3 hr, occurred in a number of dogs. When the same dose of alcohol was administered to bilaterally adrenalectomized dogs (maintained on hydrocortisone and desoxycorticosterone acetate) only a slight hyperglycemia and no significant decrease in plasma potassium occurred. The rise in hematocrit was considerably greater; the decrease in erythrocyte sedimentation rate was similar to that in the series of intact alcoholized dogs. In adrenalectomized dogs diuresis preceded oliguria: anuria did not occur in any of these dogs. Intact and adrenalectomized dogs showed some differences in control values (plasma glucose, hematocrit, erythrocyte sedimentation rate and urine output). The results of this study are in accord with the hypothesis that during acute alcohol intoxication in dogs adrenal medullary stimulation takes place causing an increase in circulating epinephrine which is largely responsible for the alcoholic hyperglycemia and hypokalemia. (Aided by a grant from the Div. of Alcohol Studies and Rehabilitation, Dept. of Health, Commonwealth of Virginia, Richmond.)

1300. TOXIC EFFECTS OF CHLORAMBUCIL (CB1348) IN LABORATORY ANIMALS. Ernest C. Hagan,* Leon Hurwitz, K. J. Davis* and Paul M. Jenner.* Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

The aromatic nitrogen mustard compound identified chemically as p-(di-2-chloroethylamino) phenylbutyric acid, is being evaluated by others as a carcinostatic agent. This drug was administered to laboratory animals in an effort to characterize some of its toxic effects. Acute oral administration to rats and guinea pigs yielded approximate LD 50's of 31 and 16.5 mg/kg, respectively. Deaths from acute doses occurred rapidly with convulsions, or in subsequent depressive states. Subacute studies using rats included 1) ad libitum feeding of a basal diet containing various levels of drug, 2) forced oral and s.c. administration of the carcinostatic agent, and 3) the use of paired feeding techniques in which the drug was incorporated in the basal diet. Prominent findings resulting from subacute administration were: 1) anorexia and inanition accompanied by weight loss in rats either fed or injected with CB1348, 2) a reduction in the total white blood cells to low values with the lymphocytes being most strongly inhibited, and with a slight shift to the left, and 3) atrophic changes in the spleen, testis, bond marrow and liver. Except for the testicular damage, recovery of poisoned animals almost always occurred after 1-month period in which the basal diet was substituted for the diet containing CB1348. (Supported by transfer of funds from the Natl. Cancer Inst. PHS.)

1301. SEROTONIN AND ACETYLCHOLINE ASSAY WITH ISOLATED TISSUES. Thomas J. Haley and James L. Leitch.* Atomic Energy Project, Univ. of California, Los Angeles.

Cambridge and Holgate (Brit. J. Pharmacol. 10:326, 1955) reported that the superfused estrous rat uterus was unsuitable for estimation of serotonin. The guinea pig ileum could be used but not in the presence of acetylcholine and histamine. The difficulties encountered appeared to be related to atropine concentration in the bathing fluid. Earlier, Rocha e Silva et al. (Brit. J. Pharmacol. 8:378, 1953) suggested that such difficulties were related to the pH of the bathing solution. Studies on the sensitivity of the superfused rat uterus and guinea pig ileum to both serotonin and acetylcholine have shown that bathing fluid pH decreases the sensitivity of the tissues to both drugs. Atropine has a similar effect and this effect is increased at a more acid pH. The rat uterus is very sensitive to serotonin and it is possible to estimate the quantity of this drug in the presence of acetylcholine by selection of a concentration of atropine which blocks the latter and only slightly reduces the response of the former. The guinea pig ileum can be used for assays but the concentration of atropine must be increased by 10. Reproducible results have been obtained in over 350 rats when the animals had vaginal smears in stage II of the estrous cycle and a body weight of 100-200 gm. The overall results obtained support the hypothesis of Gaddum and Hameed (Brit. J. Pharmacol. 9:240, 1954) that there are 2 different receptors in smooth muscle, 1 for serotonin and 1 for acetylcholine. (Performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Comm. and the Univ. of California, Los Angeles.)

1302. CHRONIC TOXICITY AND TISSUE DISTRIBUTION OF ORANGE B. Duane W. Hallesy (intr. by John Doull). Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

In a previous study (Hallesy and Doull, J. Pharmacol. & Exper. Therap. 116:26, 1956) the acute toxicity of Orange B (disodium salt of 1-parasulfophenyl, 3-ethylcarboxy, 4,4-sulfonapthoazo, 5-hydroxy pyrazole) to rats was found to be 2.5 gm/kg when given i.p. and over 15 gm/kg when given orally. To obtain information on the chronic oral toxicity of this dye, feeding studies have been initiated using male and female Sprague Dawley rats, cancer susceptible (C3H) and cancer resistant (C_{57} Br) Jackson Laboratory mice and pure bred beagle dogs. Dietary levels of 0, 1, 2 and 5% of Orange B have been fed to each species for 6 months during which time there has been no dye-induced mortality. Both the rats and dogs fed 5% Orange B in the diet exhibited periodic episodes of diarrhea but the growth rate of these animals was not markedly different from that of control animals. An extraction procedure has been devised capable of detecting microgram quantities of the dye in tissues. The tissues are homogenized in 4 N HCl and extracted with N-amyl alcohol. The alcohol portions are combined and washed 3 times with equal portions of saturated NaCl and the dye is then extracted from the alcohol by washing with 5 1-cc portions of water and determined photometrically using a Klett Summerson colorimeter with a 42 filter. Using this extraction procedure it has been found that the maximum concentration of the dye occurs in the blood within 30 min following i.p. administration of Orange B to rats. At 3 hr after the Orange B administration the dye could no longer be detected in the blood.

1303. FOLIC ACID IN AXENIC CULTIVATION OF NEMATODE CAENORHABDITIS BRIGGSAE. Eder Lindsay Hansen and Ellsworth C. Dougherty.* Kaiser Fndn. Hosp., Oakland, and Dept. of Physiology, School of Medicine, Univ. of California, Berkeley.

A recent preparation of autoclaved liver extract (ALE) used in the axenic cultivation (Dougherty and Hansen. Proc. Soc. Exper. Biol. & Med. 93:223, 1956) of C. briggsae has been found to support rapid maturation but to be inadequate for continuous cultivation since very few of the eggs hatched. The deficiency in nutrients was corrected by the addition of folic acid or the calcium salt of folinic acid. This is the first identification of a specific growth factor for this

nematode. Both chemicals were tested at levels of 0.01-100 mg/l. Folic acid was required at 1 mg/l. while folinic acid appeared to be less active. The possibility is under investigation that pteridine growth factors (Nathan, Hutner and Levin. Nature 178:741, 1956) such as those active for the trypanosomatid flagellate, Crithidia fasciculata, are involved. The reason for variations in different preparations of ALE has not yet been determined. It was apparent, however, that this recent extract, although prepared in the same general manner as previously, had a higher labile protein content as evidenced by the marked and continuing formation of a flocculent precipitate. The dry weight was almost twice that of former preparations. (Supported by grant E-926 made to the Kaiser Fndn. by the Natl. Inst. of Allergy and Infectious Diseases, PHS, and a grant from the Kaiser Fndn.)

1304. PROTECTION AGAINST FURFURALACETONE TOXICITY BY CHLOROFORM POISONING. M. G. Hardinge, R. Harvey and B. Fujikawa (intr. by Charles M. Gruber). Dept. of Pharmacology and Exptl. Therapeutics, School of Medicine, College of Med. Evangelists, Loma Linda, Calif.

The fate of furfuralacetone in the animal body is not known and parenterally administered drug has not been recovered from the bile, urine or feces. Since furfural and certain related compounds are detoxified by the liver, it was postulated that furfuralacetone is probably metabolically altered by this organ. To test this hypothesis the livers of adult male Sprague Dawley rats were destroyed by injecting s.c. 1.5 ml/kg of chloroform, diluted with an equal volume of mineral oil. Twenty-four hours later, when maximum liver destriction (66%) occurred, twice the LD_{50} of furfuralacetone (150 mg/kg) was given to control and chloroform treated animals. Control animals died within 4 hr, while chloroform treated rats survived at least 24 hr, and 75% completely recovered. Forty-eight hours after the injection of chloroform protection was still maximal. Thereafter protection steadily declined, having completely disappeared in 120 hr. This decline in protection was associated with regeneration of damaged liver tissue. When ether was substituted for chloroform no protection was observed. Prolonged feeding of high fat or high carbohydrate diets showed no protective action. Although the mechanism was not elucidated, chloroform poisoning, with associated liver damage, protected rats against lethal doses of furfuralacetone, possibly by retarding or preventing the conversion of the drug to a more toxic compound.

1305. SEPARATION OF NEGATIVE INOTROPIC AND NEGATIVE CHRONOTROPIC ACTIONS OF PROCAINE HYDROCHLORIDE ON THE BASIS OF DRUG IONIZATION. Harold F. Hardman, Walter M. Baird and Joanne I. Moore (intr. by Maurice H. Seevers). Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Procaine hydrochloride produces two outstanding effects on the isolated perfused turtle heart. One effect is to reduce the amplitude of contraction and the second effect is to produce a marked decrease in the spontaneous rate of contraction. The hypothesis is presented that the reduction in amplitude of contraction is primarily dependent on the concentration of unionized procaine present in the perfusion media. The ionized form of procaine (cation) can also contribute to this response, however, it is quantitatively much less active. The negative chronotropic response to procaine appears to be primarily dependent on the concentration of ionized procaine (cation) present in the perfusion media. The unionized form of the drug may contribute to this response when present in high concentrations. The dose of procaine cation required to produce bradycardia has been shown to be partially dependent on the pH of the perfusion media. Evidence will be presented to indicate that this effect results from an action of pH on the ionization of the cardiac receptors responsible for the negative chronotropic response to procaine. The nature of such receptors will be discussed.

1306. SYNAPTIC ACTIVITY RELATIONS OF AZACYCLONOL AND ANALOGS. E. Ross Hart, Victor H. Cohn, Jr.* and Amedeo S. Marrazzi. VA Research Labs. in Neuropsychiatry, VA Hosp., Pittsburgh, Pa.

The protective action of azacyclonol (Frenquel) against the cerebral synaptic inhibiting action of mescaline, which we have demonstrated by use of the electrocortical evoked responses in the cat, has led us to inquire into the relation of this effect to variations in chemical structure represented by the isomers and analogs of azacyclonol. Graded differences in activity are manifested. Submaximal cortical synaptic activation was effected through transcallosal association fibers and measured by recording the resulting cortical evoked potentials. The compounds were introduced into the common carotid artery on the recording side, i.e., by a relatively close arterial injection, and determinations were made of: 1) the amounts required to depress transmission, 2) the amounts which reduced or prevented (protected against) the inhibitory effect of mescaline when pitted against it. It was found that, whereas all the compounds, in sufficiently large doses, are able to impede synaptic transmission, the protective action is exhibited by varying fractions of the depressant dose - ranging from 1/20th for azacyclonol, about 1/2 for its isomer, pipradol (Meratran), and none for the analog, 1,2-diphenyl-1-(4-piperidyl)-ethanol (MER-22), which so far has not shown protection. We believe this represents the transition from a relatively non-specific synaptic depressant (similar to barbiturates) to the more specific protective action which we now associate with the tranquilizers. The ratio of protective to depressant doses can be regarded as the equivalent of the ratio of therapeutic to toxic effects, and therefore as an expression of a safety margin. The data further suggest that any stimulating properties of members of the series may be attributed, at least in part, to release phenomena resulting from the synaptic inhibitory action.

1307. PROTECTIVE EFFECT OF ANTI-HYPERTENSIVE AGENTS ON THE VASCULAR DETERIORATION ASSOCIATED WITH HYPERTENSION. Charles Heider,* Jack Burgess,* Ralph Ford,* Keith Pevey* and John Moyer. Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.

There remains a question as to the effectiveness of blood pressure reduction in arresting the vascular deterioration associated with hypertension. Two years or more is an adequate period of time to evaluate some effects of therapy in patients with severe hypertension as compared to untreated patients with hypertension of an equivalent severity. The results in our clinics have been evaluated as to clinical response in the important vascular beds, i.e., brain, heart, and kidney, which are damaged most frequently in patients with severe hypertension. Of particular quantitative significance are the observations made

on renal function, i.e., glomerular filtration rate and renal blood flow, before and after treatment in both the treated and the untreated patients. The clinical results indicate that reduction in blood pressure was accompanied by a significant over all decrease in both morbidity and mortality. The observations on renal function indicate that the patients fell into 2 groups, i.e., those with mild disease and those with severe disease. In the patients with mild hypertension, renal vascular deterioration was not significantly greater in the untreated patients than it was in the treated ones. However, in the patients with severe disease, renal vascular deterioration was quite marked over the 2-yr period. Treatment had a definite protective effect in arresting renal vascular deterioration. If uremia was present, therapy was ineffectual since adequate blood pressure reduction could not be accomplished without aggravating the renal failure.

1308. EFFECTS OF SOME CENTRALLY ACTING DRUGS ON "TREADMILL" ACTIVITY OF MICE EXPOSED TO ETHER. Max Heinrich, Jr. Dept. of Pharmacology, Univ. of South Dakota Med. School, Vermillion.

"Running time" is the time required to abolish the running ability of mice exposed to ether vapors while in a rotating bottle. The inner surface of the bottle is coated to provide traction. The drugs were administered s.c. 30 min (reserpine, amphetamine sulfate) or 1 hr (chlorpromazine hydrochloride) before exposure to ether. Reserpine and chlorpromazine may either shorten or prolong the "running time" of mice exposed to 10% ether vapor. When the bottle is rotated at 5 cm/sec reserpine (0.1 mg/kg) and chlorpromazine (0.1 mg/kg) prolong the "running time." As the dose of chlorpromazine is increased the "running time" decreases. This is especially prominent after doses which produce significant depression. The use of a slightly eccentric pulley and a belt made from rubber tubing results in an irregular rotation of the bottle. Despite the irregular motion, the average speed of the bottle is constant when determined at 5-sec intervals (av. speed 19.4 cm/sec). Pretreatment with the above doses of reserpine and chlorpromazine consistently shortens the "running time" of mice exposed to 10% ether when the rotation is irregular and the average speed 19.4 cm/sec. Pretreatment with 2 mg/kg amphetamine has no significant effect on the "running time" under these conditions. Thus, the effects of ether, as measured by the "running time," may be antagonized, unchanged, or potentiated by reserpine and chlorpromazine, depending on the conditions and the dose administered.

1309. EFFECTS OF SCH 3940 AND METHYL-PENTYNYL CARBAMATE ON ELECTRICAL ACTIVITY OF THE BRAIN: COMPARISON WITH OTHER CNS DEPRESSANTS. Charles D. Hendley. Dept. of Pharmacology, Research Div., Schering Corp., Bloomfield, N. J.

Sch 3940 is a phenothiazine derivative with behavioral effects in animals and man generally similar to chlorpromazine. It is, however, at least 7 times more potent than the latter in its effects on behavior without increase in toxicity (Irwin et al. J. Pharmacol. & Exper. Therap. in press). In the present study the electrical activity of the succinylcholine-paralyzed cat was recorded from electrodes over the cortex and in several subcortical structures, including amygdaloid nuclei, hippocampus, caudate, thalamus, hypothalamus, and tegmentum. These areas also were stimulated electrically. At a dose which varied from 0.1 to 1 mg/kg, i.v., in different animals, irregular spindles and slow waves began to appear in both cortical and subcortical recordings, while evoked responses were unchanged. Larger doses produced further slowing and spindling, but even after massive amounts (up to 50 mg/kg) fast activity was not eliminated, and the record did not resemble deep sleep or pentobarbital anesthesia. Thresholds for arousal to stimulation of the mesencephalic reticular formation were raised at a dose of 5 mg/kg. Chlorpromazine produced similar effects at the same dose levels, indicating a lack of correspondence between these measures of electrical activity and behavior. Methylpentynyl carbamate, which is used as a tranquilizer in Europe, also was studied. It slowed the EEG at a dose of 10 mg/kg, i.v., but even at 50 mg/kg a sleep record was not obtained, and the effects were different qualitatively from those of barbiturates.

1310. SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF RHODOCHLORIN, A NEW CARDIOTROPIC COMPOUND. Merrill J. Hendrickson and Herbert R. Wetherell (intr. by A. R. McIntyre). Dept. of Physiology and Pharmacology, Univ. of Nebraska, College of Medicine, Omaha.

Because the cardiotropic properties of Rhodochlorin are of the same order of potency as ouabain, a considerable quantity of this material was prepared and examined for purity by circular filter paper chromatographic technique. The R_f at 3000 sec of the dimethyl ester is 0.8685 in 10:20:70 acetone: benzene:hexane. In chloroform Rhodochlorin absorbs strongly in the ultraviolet region at 404 mu and also has a broad band with a maximum at 246.5 mu. The peaks in the visible spectrum are at 667, 611.5, 559, 530, 500 and 471. The order of intensities are: I, V, II, IV, VI, III. The infrared spectrum shows, in addition to many peaks in the range 600-1600 cm^{-1}, two prominent absorption bands in the carbonyl region at 1700 and 1738 cm^{-1}. Rhodochlorin dimethyl ester forms curious lenticular crystals as well as aggregates of needles in n-propanol. The melting point of the dimethyl ester is 208°C. The HCl number of Rhodochlorin is 10, and the presence of two free carboxylic groups is indicated by extraction from ether with buffers. Free Rhodochlorin has only slight solubility in all the usual organic solvents except pyridine and formic acid. The cardiotropic activity of Rhodochlorin is decreased in those derivatives with side chains on the gamma carbon and by loss of the vinyl group of free carboxyls at positions 6 and 7, while the presence or absence of a formyl radical at position 3 has very little effect. The biological activity resembles that of the cardiac glycosides and is capable of restoring contractility to a heart in standstill perfused with a calcium-free solution. The "recoupling" of electrical action to muscle shortening is strikingly similar to that seen with Ca^{++}. Unlike the cardiac glycosides the material is relatively nontoxic.

1311. MINIATURE TRANSDUCER FOR ELECTROMAGNETIC DETERMINATION OF BLOOD FLOW IN UNANESTHETIZED ANIMALS. G. Herrold, A. Kolin, N. S. Assali and R. Jensen (intr. by D. B. Taylor). Univ. of California, Los Angeles.

Determination of blood flow in unanesthetized animals by means of the electromagnetic flowmeter (Kolin. Proc. Soc. Exper. Biol. & Med. 35:53, 1936 and 46:235, 1941) has an inherent difficulty which is not present in work with anesthetized animals. Motion of the animal leads to displacement of the blood vessel relative to the magnetic field which, as a rule, results in signals that interfere with the

measurement of flow. This source of disturbance has been eliminated by fixing the pick-up channel to the magnet which is small enough to be implanted at the site of the blood vessel. The magnet–pick-up assembly for the carotid artery (diameter 2.5 mm) of a dog weighs 11.5 gm. This includes the weight of the nonirritant plastic (methyl metacrylate) into which the assembly of magnet and pick-up electrodes is molded. The wire leads to the magnet windings and to the pick-up electrodes are enclosed in polyvinyl tubing which is molded into the solid plastic. The miniature magnet core (approximately 15 x 9 x 3 mm) is made of silicone steel laminations. The two coils have 700 turns of gauge 30 wire each. The magnet is energized with 60 cycle a.c. The circuits and recording equipment used were as described in previous papers (Kolin. Rev. Sci. Instr. 23:253, 1952). The magnetic field obtained with a current of 65 mA is 750 oersteds. The sensitivity used in initial experiments (considerably below the maximum possible value) was 4 mm/cc/sec. Records free of disturbance have been obtained with unanesthetized dogs in a standing position during mastication, attempts to jump in response to painful stimuli, and moving in response to startling stimuli. (Supported by grants from the Office of Naval Research, the Los Angeles Heart Assoc. and PHS Grant 1755 C3.)

1312. PHARMACOLOGY OF DIAMMONIC ESTERS. John Hidalgo* and Victor P. Seeberg. Research Div., Cutter Labs., Berkeley, Calif.

Forty-nine, 2-ammonioethyl 3-ammoniopropionate salts were studied in mice for toxicity, in vitro for cholinergic activity on isolated rabbit ileum, and in rats for hypotensive activity. The toxicity varied widely and did not appear related to cholinergic or hypotensive activity. The cholinergic activity was decreased by substitutions on the nitrogen of the ethyl radical but not by substitutions on the nitrogen of the propionate. Substitutions on the carbons of the propionate abolished cholinergic activity but substitutions on the carbons of the ethyl only decreased it. The compounds have an internitrogen distance close to that of hexamethonium and, as expected, were ganglionic blockers as shown by the inhibition of the response to DMPP in vitro and in vivo. The duration of the hypotensive action, when present, varied widely, the most active compound being 1-methyl-2-(methylpyrrolidinio)-ethyl 3-)1-methylpyrrolidinio)-propionate diiodide, while 2-(1-methylpyrrolidinio)-ethyl 3-(1-methylpyrrolidinio)-propionate diiodide was far less active. Hydrolysis of the ester bond by plasma cholinesterase or liver brei did not occur in vitro. Injections into the portal circulation of monkeys were as effective in lowering the blood pressure as peripheral injections. Orally the compounds were effective in inducing relaxation of the nictitating membrane of dogs. Some sedation was noted in the dogs receiving the active ester compounds.

1313. EFFECTS OF DECREASING BLOOD VOLUME UPON PRESSOR AND DEPRESSOR RESPONSES. James G. Hilton, Dept. of Pharmacology, Univ. of Mississippi Med. Ctr., Jackson.

A study of the effects of decreasing the blood volume of anesthetized dogs on the blood pressure responses to epinephrine and histamine has been carried out in the following manner. The animals were anesthetized with pentobarbital sodium and the blood volume was determined by the use of Evans Blue dye. On completion of the determination of the blood volume, a series of graded doses of either epinephrine or histamine were administered and the effects of each dose of the selected drug on the blood pressure were recorded. Following the largest dose in this series, the animal was bled 10% of the previously determined blood volume. After the blood pressure had stabilized at a new control level, the series of graded doses of epinephrine or histamine were repeated and the blood pressure responses were recorded. The records from these experiments were analyzed in terms of the maximum or minimum blood pressure attained, the actual rise (pressor response) or the actual fall (depressor response) in blood pressure and the duration of the rise or fall in blood pressure before and after bleeding. As the result of these experiments, it was found that the maximum or minimum to which the blood pressure would rise or fall was significantly affected by the change in blood volume but the pressor or depressor responses and the duration of the responses were not significantly altered by the change in blood volume. (Supported by a grant from the Natl. Heart Inst., PHS.)

1314. EFFECT OF SUCCINYLCHOLINE ON CARDIAC ARRHYTHMIAS. Philip Hitchcock, Joseph R. DiPalma and Alfred J. Catenacci.* Depts. of Anesthesiology and Pharmacology, Hahnemann Med. College and Hosp., Philadelphia, Pa.

Use of petroleum ether administered by vaporization and i.v. doses of epinephrine to produce cardiac arrhythmias in the dog has previously been described from these laboratories. Using this test system, we have found that the relaxant drug, succinylcholine, tends to prevent the development of the arrhythmias. The doses of succinylcholine vary widely, from 0.25 to 50 mg/kg. The duration of the effect varied from 5 min to over 2 hr according to the amount of succinylcholine given. The dose of epinephrine required to produce arrhythmias after succinylcholine varies from 2 to 8 times the control dose. This action of succinylcholine is not entirely constant and in about 20% of experiments has not been seen. The effect is not prevented by atropine. In this respect it is similar to the previously described action of acctylcholine. We do not believe that this is a ganglionic effect since other ganglionic blocking agents, tetraethylammonium chloride, hexamethonium and Arfonad do not have a similar effect, nor do they modify this action of succinylcholine. (Supported by a grant from Abbott Labs.)

1315. MECHANISM OF INTESTINAL ABSORPTION OF DRUGS. C. Adrian M. Hogben,* Lewis S. Schanker* and Bernard B. Brodie. Natl. Heart Inst., Bethesda, Md.

The following results are compatible with a hypothesis that intestinal absorption of drugs is due to diffusion of the unionized drug across a lipoid barrier and that it is not necessary to postulate specific transport mechanisms. When the entire small intestine of the anesthetized rat is perfused with drugs dissolved in saline, acidic drugs are rapidly absorbed if their pKa is above 2.5 and basic drugs rapidly absorbed if their pKa is below 8.5 If the intestine is perfused with buffered solutions, pH 4 to 8, absorption of acidic drugs decreases progressively and absorption of bases increases progressively with increasing pH. Over this range of physiological pH, absorption of many drugs changes several-fold. The ratio of concentration of drug in plasma to concentration in intestinal lumen was measured when the drug was intravenously administered and simultaneously perfused through the intestine. The concentration ratio at which there is neither absorption nor secretion is a function of the pK of the drug. Assuming that drugs transfer only in their undissociated

form, the plasma to gut ratio of acids is higher and that of bases lower than that predicted from the pH of the solution leaving the intestine. These differences would be comprehensible if the absorbing surface has a slight permeability to ionized drug and if it is exposed to a pH of about 5. This virtual pH provides a basis for understanding rapid absorption of those acids which are largely ionized.

1316. POSSIBLE MECHANISM OF ACTION OF QUINIDINE. W. C. Holland. Dept. of Pharmacology, Vanderbilt Med. School, Nashville, Tenn.

Quinidine at a concentration of 7.8×10^{-5} gm/ml increases the refractory period and depresses both the rate and amplitude of beat of isolated rabbit auricles. These effects can be enhanced by elevating the K content of the medium 2-fold and almost completely blocked by lowering the K to 1/16 the normal amount. These observations suggested that quinidine produced its effects by depressing the outward flux of K ions. Ion transport studies revealed this to be the case. The inhibitory action on the net outward movement of K ions was small but significant when the outward flux was accelerated 2- to 3-fold by the addition of acetylcholine (3.0×10^{-5} gm/ml) to the medium, the depressant effect of quinidine on K loss was much more pronounced. In all these studied the auricles were stimulated at a constant rate (200/m) so as to eliminate effects that might occur as a result of rate changes induced by quinidine and acetylcholine. There is considerable evidence at hand which suggests that the acetylcholine system is an integral component of an ion-carrier system that increases the passive flux of cations and certain anions across the cell membrane during the passage of the cardiac action potential. Furthermore, it is known that quinidine has anti-vagal and atropine-like properties. These studies suggest that in final analysis quinidine produces its pharmacological action by blocking the action of intracellularly released acetylcholine.

1317. COMPARISON OF PYRETOGENIC ACTION OF 5-HYDROXYTRYPTOPHAN (HTP) AND LYSERGIC ACID DIETHYLAMIDE (LSD). Akira Horita and John H. Gogerty (intr. by J. M. Dille). Dept. of Pharmacology, School of Medicine, Univ. of Washington, Seattle.

The central excitatory actions of 5-hydroxytryptophan (HTP), the precursor of serotonin, in various animals have been described by several investigators. In most of these reports the similarity between these actions of HTP and lysergic acid diethylamide (LSD) has been mentioned. Since we have found that one of the most consistent and measurable actions of LSD in laboratory animals is its pyretogenic effect, studies were carried out to determine whether HTP shared in addition to its excitatory actions the pyretogenic effect of LSD. Various dose levels of HTP were given to both normal rabbits and rabbits pretreated with Marsilid phosphate, an amine oxidase inhibitor. In the untreated animals doses of HTP as high as 75 mg/kg i.v. produced no rise in rectal temperature. In the Marsilid pretreated rabbits doses of HTP from 30 mg/kg to 50 mg/kg exerted a marked pyretogenic effect, the higher doses often leading to death of the animal at the height of the hyperthermia. The pyretogenic effect of HTP administered 2 hr after reserpine and Marsilid appears to be potentiated. This potentiation is also seen in the pyretogenic action of LSD. These results are of interest in view of the recent hypotheses concerning the role of serotonin in the mechanisms of action of LSD and reserpine.

1318. CONJUGATION AND EXCRETION OF AMINOBENZOIC ACIDS. K. C. Huang* and P. K. Knoefel. Dept. of Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.

The 3 isomeric aminobenzoic acids were given intravenously to dogs in chloralose anesthesia. Urine, bile and blood plasma were collected, fractionated with Dowex-50 columns and analyzed by diazotization and coupling. Synthesis of conjugates was calculated from excretion and plasma concentration; renal tubular transport from excretion and filtration (creatinine clearance). Glucuronide synthesis was about equal with the 3 isomers, and greater than hippurate synthesis, except for the para isomer, which formed hippurate more rapidly than glucuronide. Renal tubular transport was positive (secretion) for the ortho glucuronide, the para hippurate, and the metahippurate (at low plasma concentrations only); negative (reabsorption) for the meta and para glucuronides, the orthohippurate, the meta hippurate (at high concentrations).

1319. NATURE OF SEROTONIN BINDING IN TISSUES. F. B. Hughes,* R. Kuntzman* and P. A. Shore. Lab. of Chem. Pharmacology, Natl. Heart Inst., Bethesda, Md.

The localization of serotonin in gastrointestinal mucosa and in brain, tissues rich in monoamine oxidase activity, has indicated that the indole is normally present in a bound form. Attempts to demonstrate by direct means the presence of a serotonin complex in homogenized tissues have been unsuccessful. Thus, dialysis of homogenates of intestines or brains from iproniazid-treated rabbits has failed to indicate the presence of undialyzable serotonin; the serotonin content of tissues rapidly diminishes following homogenization, suggesting that serotonin is not present in the homogenate as a firmly bound, protected complex; differential centrifugation of a brain homogenate has failed to indicate a particular affinity of serotonin for any of the cell fractions. Alternative explanations of the nature of serotonin binding will be discussed.

1320. HEMODYNAMIC RESPONSES TO RESCINNAMINE (MODERIL). D. E. Hutcheon, V. Kozler* and D. Morris.* Research Labs., Chas. Pfizer and Co., Inc., Brooklyn, N. Y.

Rescinnamine was reported by Cronheim et al. (Proc. Soc. Exper. Biol. & Med. 86:120, 1954) to have hypotensive and other cardiovascular properties similar to reserpine in anesthetized dogs. The present studies extended the comparison of reserpine and rescinnamine effects in rabbits and dogs. Rabbits were injected intraperitoneally with rescinnamine or reserpine (12-108 μg/kg daily). On the 5th day the 25 rabbits used in the experiment were anesthetized with urethane and the carotid arterial blood pressure was recorded. Both drugs lowered the arterial blood pressure without significantly slowing the cardiac rate. The average blood pressures of the controls and the rabbits injected with daily doses of 12, 36 and 108 μg/kg rescinnamine were 94, 85, 80 and 65 mm Hg, respectively. No significant difference in the hypotensive activity of reserpine and rescinnamine was observed. Rabbits on the higher doses of both drugs developed miosis and diarrhea 3 days after treatment was begun. Both drugs lowered the arterial blood pressure for several days when injected intravenously in a single dose (50-400 μg/kg) in conscious dogs. In some dogs the blood pressure rose temporarily prior to the prolonged hypotensive phase. A decrease in cardiac rate paralleled the fall in blood pressure.

The dose causing a 25% drop in blood pressure in 24 hr was estimated to be 65 μg/kg rescinnamine and 90 μg/kg reserpine although the difference in activity was not significant ($P > 0.1$). The cardiac output, determined by the dye dilution method in dogs injected 18 hr previously with 50 μg/kg rescinnamine, intravenously, was low compared to the controls. The decrease in minute volume was believed to be partly due to cardiac slowing and partly to a reduced venous return.

1321. STIMULATION OF RESPIRATION BY 2-METHYLAMINO-6-METHYL HEPTANE. David C. Jerram,* Berwin Monroe,* Frederick Spencer* and Lloyd D. Seager. Dept. of Physiology and Pharmacology, Univ. of Arkansas Med. Ctr., Little Rock.

Stimulation of respiration by aliphatic amines has been variously reported by many authors. In the investigation of a series of aliphatic amines, we have noted that the intracarotid injection of small doses of 2-methylamino-6-methyl heptane markedly increases respiratory rate and volume in dogs anesthetized with pentobarbital. Injections of the drug into an unoperated, anesthetized dog have a more marked effect when the animal is under deep anesthesia than when under light anesthesia. In experiments designed to determine the mechanism of action, we have found that serial i.v. injections of 5 mg/kg of the drug produce a diminishing response. Recovery of responsiveness occurs and is greater the longer the interval between injections. Initial injections increase respiratory rate and volume 200% or more, while subsequent injections may show less than a 100% increase. Removal of the aortic arch and carotid receptors before or during the series of injections has little or no effect on the expected response to the drug. This would seem to indicate that stimulation of respiration by 2-methylamino-6-methyl heptane is primarily a direct central action.

1322. PHARMACOLOGIC ACTION OF VITAMIN A ON THE SKIN AFTER INTRACUTANEOUS INJECTION. Howard A. Jewell,* Harry Taube,* Maude E. Nicholls* and Robert A. Lehman. Research Labs., Campbell Pharmaceutical Co., and Dept. of Therapeutics, New York Univ. College of Medicine, New York City.

Recent reports (Steinberg, 1954, Mullens, 1956) have indicated that the human wart papilloma may be dramatically resolved by injection of vitamin A directly into the lesion. These findings are unexpected since attempts to treat verrucae with systemic vitamin A have been equivocal. The present experiments were therefore undertaken in an effort to clarify the mechanisms involved. An especially formulated aqueous solution of crystalline vitamin A palmitate, Keramin Injection (Steinberg's Solution), containing 50,000 IU/cc was injected intracutaneously in doses of 0.03 cc into the skin of rabbits. The animals were killed after 1, 2, 4, 7, 14, 21 and 28 days. On microscopic examination, the epidermis was found to be altered markedly. Whereas normal rabbit epidermis consists of one or two layers of cells with loose strands of overlying corneum, after injection of vitamin A, the epidermis showed 5-10 times this number of cellular layers in various stages of differentiation with a firmly adherent, dense corneum. The basal cells were small and definite squamous cellular elements were present including a stratum granulosum. Nitotic figures were rare. The effect reached a peak 4-7 days after an injection and then declined slowly. Suitable controls of normal skin and skin injected with Keramin vehicle were used for comparison. The results suggest that there is a slowing down in the rate of maturation and destruction of the epidermal cells and that this is in some way responsible for the regression of injected verrucae observed in the clinic.

1323. FACTORS AFFECTING RESPONSE OF ISOLATED RABBITS' AURICLES TO ADENOSINE TRIPHOSPHATE. E. Jones* and W. C. Holland. Dept. of Pharmacology, Vanderbilt Med. School, Nashville, Tenn.

We have been impressed by the similarity of the affects of acetylcholine (ACh) and adenosine triphosphate (ATP) on isolated rabbit atria. Both produce a transient depression of the rate and amplitude of beat. Recently it has been observed that they have similar action on the membrane resting and action potential of guinea pig atrial fibers (Johnson and McKennon, Nat. 178: 1174, 1956). These effect on rate and amplitude of beat are enhanced by physostigmine and blocked by quinidine. However, there are some striking differences. The effects of ACh, but not those of ATP, were reversed or prevented by atropine. Whereas the response of auricles to ATP is enhanced by Mg^{++}. We have also observed that the effects of these drugs on isolated atria are affected in a comparable and characteristic manner by changes in the Na^+ and K^+ content of the medium. The action of ATP is extremely sensitive to these variations. For example, lowering the Na^+ content to 1/2 completely blocks the action of ATP and modifies only slightly the ACh effect, whereas lowering the K^+ enhances the response to both drugs about equally. These findings suggest that both drugs produce their inhibitory effects by increasing the outward flux of K^+ and the inward flux of Na^+. However, the 2 substances probably have different sites of action on the fundamental mechanisms controlling passive movement of cations, or it is possible that ATP produces its effect by releasing ACh at an intracellular site from a bound form. This endogenous ACh reacts with receptors not available to blockade by atropine; possibly because of existing permeability barriers.

1324. ARSENIC LEVELS FOLLOWING PROLONGED ORAL ADMINISTRATION OF POLYBENZARSONE [BIS-(2-HYDROXY-5-ARSONOPHENYL)-METHANE] TO DOGS. W. R. Jones,* J. W. Newberne,* R. B. Forney, R. W. Begley,* W. M. Alexander,* L. C. Weaver and B. E. Abreu. Research Dept., Pitman-Moore Co., and Pharmacology Dept., Indiana Univ. Med. School, Indianapolis.

Arsenic was determined by a modified Guthzeit procedure using wet digestion. Levels were determined for blood, urine, idney and liver at bi-weekly intervals in dogs receiving polybenzarsone in doses of 100 and 200 mg/kg/day for 2-4 wk. Additional dogs received the above doses for 4 wk followed by identical arsenic determinations 2 and 4 wk following cessation of drug administration. Arsenic levels during the intervals of drug administration exhibited the following ranges for 100 and 200 mg/kg/day, respectively: a) blood, 0.0-0.04 and 0.03-0.18 mg/100 cc; b) urine, 0.06-0.66 and 1.83-9.92 mg/l.; c) liver, 0.11-7.30 and 2.16-9.55 mg/100 gm tissue; and d) kidney, 0.35-8.56 and 1.08-12.70 mg/100 gm tissue. The upper limits of the arsenic levels occurred at the end of 4 wk except for tissue levels following 200 mg/kg/day dose which were observed at the end of 2 wk. It is apparent that a 2-fold increase in dosage produced a several-fold increase in arsenic levels. Arsenic levels had reached or were approaching control 4 wk after stopping drug. During the study, polybenzarsone produced

no significant alteration in weight, blood cell counts (red, white and differential), hemoglobin, hematocrit or organ function (liver and kidney). Histopathological examination at necropsy revealed only perceptible kidney damage. Rats were given doses of 50, 100, 200, and 400 mg/kg/day for 8 wk without producing any histopathological alterations or effect on growth.

1325. CONTINUOUS RECORDING OF EXPIRATORY $C^{14}O_2$ FROM ACETATE AND GLUCOSE IN NORMAL AND TUMOR MICE. Jon J. Kabara,* George T. Okita and George V. LeRoy.* Argonne Cancer Research Hosp., and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

Previous isotopic studies dealing with the oxidative metabolism of neoplastic tissue have been primarily conducted with in vitro systems. In the experiments to be reported a continuous recording system (LeRoy, Okita, Tocus. In preparation) for measuring radioactive expiratory carbon dioxide was used. With this instrument both normal and Ehrlich carcinoma CF-1 female mice were studied in regard to their ability to oxidize radioactive acetate and glucose to carbon dioxide. The results were compatible with other investigators (Weinhouse. Adv. Cancer Res. 111: 1955) who suggest a high rate of glycolysis in tumor tissue as reflected by an increased ability to oxidize glucose and an impaired capacity to oxidize a short-chain fatty acid. As measured by acetate-1-C^{14} oxidation curves a shift of oxidative capacity in the whole animal toward the "tumor pattern" is evident as early as 48 hr after tumor inoculation. The differences observed between normal and tumor mice could not be explained on the basis of an altered carbon dioxide metabolism since injected carbon-14 sodium bicarbonate in control and tumor mice gave expiratory CO_2 turnover times and rates that were similar. Also, extrapolation of their respective CO_2 regression curves to time zero gave miscible pool figures that were statistically identical. The difficulties and limitations of interpreting expiratory data will be discussed.

1326. EARLY RESPONSE OF UTERINE SODIUM AND CHLORIDE TO ESTROGEN ADMINISTRATION. Sumner M. Kalman. Dept. of Pharmacology, Stanford Univ. School of Medicine, San Francisco, Calif.

Previous work done here showed that radioactive sodium entered the uterus of the spayed rat after estrogen injection to a greater degree than could be accounted for by the increased water content (J. Pharmacol. & Exper. Therap. 115:442, 1955). The present study was undertaken to see whether uterine total sodium changed appreciably and to try to relate any such change to the total potassium, chloride and water in this tissue. At hourly intervals up to 6 hr after ethinyl estradiol administration to adult, spayed rats, the animals were killed and determinations of Na, K, Cl, and H_2O were performed on each uterus. The results showed that the sodium space started to enlarge more rapidly than the chloride space about 3 hr after estrogen treatment. For example, on one experiment where 5 rats were used in the control group and in each of the hourly groups the mean sodium content was 67 m.e./kg for the controls and 95 m.e./kg for those rats which had received estrogen 4 hr previously. Corresponding chloride values were 65 m.e./kg for controls and 71 m.e./kg for the 4-hr group. All values are expressed as m.e. per kg wet uterine weight. The following conclusion appears consistent with the data obtained: one early result of estrogen administration to ovariectomized adult rates is the extension of the uterine sodium space to a degree not shared by the chloride space.

1327. URINARY METABOLITES OF ATROPINE AS A FUNCTION OF DOSAGE. Sarah C. Kalser (intr. by J. H. Wills). Pharmacology Branch, Directorate of Med. Research, Army Chem. Ctr., Md.

Ad the dose of atropine, labeled with C^{14} on the alpha carbon of the tropic acid moiety, given to mice is increased from 3 to 80 mg/kg, there are changes in the relative amounts of the major excretory products appearing in the urine during the 24 hr following atropine administration. Contrary to expectation, the component tentatively identified as unchanged atropine does not undergo the expected tremendous increase at higher dosage levels. It does increase definitely but not to an extent that would imply that detoxification systems are saturated. Hydrolysis of atropine to tropic acid does not increase at the higher doses and remains a very minor detoxication pathway. The outstanding change seen with increasing dose of atropine is that the major product in the urine becomes a substance with an rf of 0.58, whereas at the 3 mg/kg dose of atropine the major urinary product has an rf of 0.24. A component with an rf of 0.75 also increases in amount as the atropine dose increases. None of the metabolites has been identified positively, but it is hypothesized that the component decreasing in relative importance as the dose increases may be a glucuronide and that those which increase in importance may be oxidation products of atropine.

1328. RENAL TUBULAR SECRETION OF POTASSIUM AND BASIC ORGANIC COMPOUNDS. Alexander Kandel and Floyd R. Domer.* Dept. of Pharmacology, Tulane Univ. School of Medicine, New Orleans, La.

In a previous report Kandel (Fed. Proc. 15:1446, 1956) showed a competition between potassium and basic organic compounds for renal tubular secretion, as well as an increased capacity for renal tubular secretion of basic organic compounds in dogs force-fed potassium chloride. Current investigations reveal a similar competition between potassium and mecamylamine (3-methylaminoisocamphane), as well as an increase in the renal tubular secretion of the latter in the potassium chloride-conditioned dog. The increased clearance of potassium evoked by administration of a carbonic anhydrase inhibitor, acetazoleamide (2-acetylamino-1,3,4-thiadiazole-5 sulfonamide) is reduced by several basic organic compounds. The significance of this influence on the renal tubular secretion of potassium by N'methylnicotinamide; Darstine, 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol methobromide; Priscoline, 2-benzimidazoline and the basic cyanine dye (#863), 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride will be discussed. (Supported by a research grant from the Louisiana Heart Assoc.)

1329. RATE OF COLLAGEN FORMATION IN RAT BIOPSY-CONNECTIVE TISSUE. Kung-Ying Tang Kao and Robert J. Boucek (intr. by J. L. Radomski). Howard Hughes Med. Inst., Miami, Fla.

The rate of collagen formation in biopsy-connective tissue obtained from male rats was followed by the simultaneous implantation of 3 sponges along the dorsum and subsequent single removal weekly. Series of animals were implanted so that tissue could be removed at 3-day intervals from days 7 to 25. A group of animals were permitted to develop connective tissue in the sponge for 100 days. The sponge-biopsy was separated into saline-soluble and saline-

insoluble fractions and nitrogen, hydroxyproline and hydroxylysine were determined chemically. Total protein content (gm protein per gm sponge) of the biopsy-tissue increased from 0.6 at 7-14 days to 0.9 at 100 days. Early (7-14 days) 38% of protein was found in the saline-insoluble fraction while later (100 days) this was increased to 63%. Collagen content calculated from hydroxyproline increased rapidly during early growth. Values in mg/gm sponge for days 7, 10, 14, 17, 21 and 100 were 11, 37, 85, 113, 157 and 385, respectively. Approximately 50% of the collagen from biopsy-connective tissue was formed during the first 21 days of tissue growth. The percentage of collagen in the saline-insoluble fraction increased from 5.2 at day 7 to a relatively constant value of 45.8 at day 21. Hydroxylysine was found at the 10th day of tissue growth and reached a constant concentration by day 21. (Supported in part by Grant H-1716, Natl. Insts. of Health, PHS.)

1330. RE-EVALUATION OF PHARMACOLOGICAL ACTIONS OF MYTOLON. Alexander G. Karczmar and John J. Fudema.* Dept. of Pharmacology and Exptl. Therapeutics, Stritch School of Medicine of Loyola Univ., Chicago, Ill.

Mytolon was described as a curaremimetic agent; it was further suggested that its cholinergic side actions were due to its antiacetylcholinesterase activity, one-third that of neostigmine (Hoppe. J. Pharmacol. & Exper. Therap. 100:333, 1950). It was shown at present that, in dogs and cats, Mytolon potentiates muscarinic and, following atropine, nocotinic hemodynamic responses to acetylcholine at AED_{50} dose of 1 μg/kg, and responses to faradic stimulation of peripheral vagus at AED 50 dose of 3.5 μg/kg. These doses are lower than facilitatory doses of neostigmine and 25-100 times lower than Mytolon ED_{50} dose for paralysis of neuromyal junctions. Mytolon also increases many times toxicity of neostigmine in mice (Lands et al. J. Pharmacol. & Exper. Therap. In press). Mytolon could therefore relax musculature not as a curaremimetic but as an anticholinesterase agent via acetylcholine accumulation. This is consistent with poor antagonism between Mytolon and neostigmine at neuromyal junctions. However, small doses of Mytolon (10 μg/kg) sensitize the skeletal muscle to d-tubocurarine; moreover, vagus blockade by Mytolon (50-100 μg/kg) is easily antagonized by neostigmine. Experiments were run to study, following administration of Mytolon: 1) inhibition of acetylcholinesterase in vivo: special procedure was employed to avoid reversal of inhibition during measurement; 2) acetylcholine accumulation and fate, on i.v. administration; 3) electric activity at the neuromyal junction; and 4) interactions of Mytolon with other agents for which a site of action distinct from that of d-tubocurarine was suggested (Karczmar. J. Pharmacol. & Exper. Therap. 118: no. 4, 1956). Data obtained indicate that neither curaremimetic nor anticholinesterase mechanism of action can entirely explain the neuromyal activity of Mytolon.

1331. CENTRAL RESPIRATORY DEPRESSANT EFFECT OF "HEMICHOLINIUM." Y. Kase* and H. L. Borison. Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

Schueler has described "hemicholinium" compounds which possess a central toxic action on respiration (J. Pharmacol. & Exper. Therap. 115:127, 1955). Forty cats were employed in the present study. In unanesthetized cats, 1.0 mg/kg intravenous hemicholinium (HC-3) produced respiratory paralysis without loss of consciousness within 2 hr. The stereotaxic technique was used in pentobarbitalized cats for electrical stimulation of the medullary inspiratory substrate. Prior to cessation of breathing after HC-3, pneumographic recording showed irregular and gasp-like respirations, especially in thoracic activity. Phrenic nerve stimulation continued to be effective after respiratory paralysis. Intravenous HC-3 evoked an immediate and transient fall in blood pressure but the level decreased only slightly after recovery from the initial drop. The effect of 1.0-5.0 mg/kg HC-3 on centrally evoked inspiratory responses was characterized by an increase in the stimulus threshold for sustained inspiration and a decrease in amplitude of responses to submaximal stimulation; however, inspiratory responses to maximal stimulation were not significantly diminished, even after cessation of spontaneous respiration. Concomitant centrally evoked vasodepressor responses as well as carotid occlusion pressor responses were reduced appreciably by the drug. Vagotomy did not modify the effects of HC-3. Decerebrate rigidity was reversibly inhibited by sublethal doses of HC-3. The respiratory paralyzant dose of HC-3 bt the intracarotid route was approximately one-fifth that required by the intravenous route. Thus, HC-3 depressed the vital regulatory functions of the lower brain stem. (Supported by Grant No. B-491, Natl. Insts. of Health, PHS.)

1332. INCREASED VENTILATION AFTER PENTOBARBITAL IN MAN. Arthur S. Keats and Yoshio Kurosu.* Dept. of Anesthesiology, Baylor Univ. College of Medicine, and Jefferson Davis Hosp., Houston, Tex.

Westfall (J. Pharmacol. & Exper. Therap. 101:163, 1951) reported that low concentrations of several barbiturates, including pentobarbital, increased the oxygen consumption of rat brain slices. High concentrations depressed oxygen consumption. He postulated that sedative doses of barbiturates in man should increase brain oxygen consumption. Several investigators have failed to observe depression of respiration following small doses of pentobarbital and thiopental. The data of Keats and Beecher (J. Pharmacol. & Exper. Therap. 105:109, 1952) even suggest that respiration is increased by small doses of pentobarbital. Seven normal subjects received intramuscular injections of each of the following drugs per 70 kg of body weight in random order at 4-day intervals: saline, 100 mg pentobarbital sodium, 200 mg pentobarbital and morphine 10 mg. Alveolar pCO_2 and alveolar ventilation were measured before and after each drug while subjects breathed room air and 3% CO_2 in O_2. Changes in ventilation were measured by left or right shifts of the stimulus-response curve (pCO_2 vs alveolar ventilation). Saline produced no significant change in respiration. Pentobarbital 100 mg significantly increased ventilation. Pentobarbital 200 mg significantly decreased ventilation, although not as much as morphine.

1333. DISTRIBUTION OF OXYTOCIN, VASOPRESSIN AND INTERMEDIN IN HYPOPHYSIS OF THE BEAVER. Frances O. Kelsey, Lowell Sorensen* and H. J. Clausen.* Univ. of South Dakota School of Medicine, Vermillion.

A study of hormone distribution in the beaver (Castor canadensis) was undertaken because it has been shown that there is no discrete intermediate lobe in this species (J. Pharmacol. & Exper. Therap. 116: 34, 1956). The animals were decapitated under ether anesthesia and the glands separated into anterior and neural lobes and stalk. The separated portions were placed in acetone, and later dried and extracted in 0.25% acetic acid. The extracts were assayed for

oxytocic activity using the anesthetized fowl, for pressor activity using the anesthetized dog or cat, for antidiuretic activity using the hydrated unanesthetized rat and for melanophore expanding activity using the hypophysectomized frog. Oxytocic, pressor and antidiuretic activity were restricted to neural lobe and stalk extracts while melanophore expanding activity was found only in anterior lobe extracts. The relation of oxytocic to pressor and antidiuretic activity in the neural lobe extracts was approximately that of standard posterior pituitary powder.

1334. PHARMACOLOGY AND TOXICOLOGICAL ACTIONS OF MAGNESIUM ARSENATE PHOSPHOR. M. L. Keplinger* and William B. Deichmann. Dept. of Pharmacology, Med. School, Univ. of Miami, Coral Gables, Fla.

Magnesium arsenate phosphor, a luminescent chemical, was studied to determine its pharmacological and toxicological properties. Administered in aqueous carboxymethylcellulose suspension, the appropriate lethal oral dose was found to be 4.7 gm/kg in mice, 280 mg/kg in rats and 80 mg/kg in rabbits. The approximate lethal intraperitoneal dose was 2.1 gm/kg in rats. Signs of intoxication, which began about 24 hr after administration and continued for several days, include lethargy, anorexia, loss of body weight, gastroenteritis and severe diarrhea with watery stools. Oral administration to dogs in doses of 170 and 184 mg/kg caused nausea and profuse vomiting 30 min after administration. After injection s.c. into dogs in doses of 210 and 308 mg/kg and i.p. into rats in a dosage range from 0.037 to 10.7 gm/kg, there was limited toxicity and most of the material was found as a composite mass at the site of injection when the animals were killed 5 days after administration. Thus the material is obviously poorly absorbed. The i.m. injection into dogs in doses of 210, 268 and 308 mg/kg caused a marked local irritant effect, pain, massive edema in the injected limb and extensive tissue damage. One dog developed a superficial, cutaneous ulcer about 4 cm in diameter. Gross pathological examinations of rat, mouse and rabbit organs revealed distended cutaneous vessels, hemorrhagic, irritated intestines with mucosal sloughing and intestines filled with watery fluid. (Supported by a grant from Sylvania Electric Products, Inc.)

1335. ACTION OF CIGARETTE SMOKE ON CARDIOVASCULAR HEMODYNAMICS. Gerald A. Kien,* Norman Lasker* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Illinois, College of Medicine, Chicago, Ill.

There is considerable controversy concerning the action of cigarette smoke on cardiovascular hemodynamics. This study was designed to determine such actions of cigarette smoke in the open-chest pentobarbitalized dog in which blood pressure, cardiac output, coronary blood flow, cardiac oxygen consumption, cardiac work and the electrocardiographic effects were measured. Immediately following the administration of 1200-1500 cc of cigarette smoke, making use of a standard "king sized" nonfiltered cigarette, the smoke being administered by a specially calibrated smoking device, there was a brief but marked slowing of the heart followed by a sustained pressor response. These effects were attributed to autonomic ganglionic stimulation by the absorbed nicotine from the cigarette smoke. The time course of the hemodynamic responses indicated an early increase in cardiac work which extended over a 3-min period. The coronary blood flow was elevated in relation to the rises in both blood pressure and cardiac output. No independent action of cigarette smoke on the coronary vessels was observed. The coronary arteriovenous oxygen difference decreased markedly at first. This was then followed by a prolonged increase in the coronary arteriovenous oxygen difference. As a consequence, initially the cardiac oxygen utilization was reduced during the period of a greatly elevated cardiac work. This decrease in cardiac oxygen utilization was then followed by a sustained increase. These alterations may be explained on the basis of metabolic changes in the myocardium. That such effects are detrimental to adequate cardiac function is suggested by the extreme electrocardiographic alterations incident to the changes in oxygen utilization during the period of elevated cardiac work. (Supported in part by a research grant from the PHS.)

1336. MECHANISM OF HEMOLYTIC ANEMIA INDUCED BY NITROFURANTOIN (FURADANTIN). Exall L. Kimbro, Jr., Marie V. Sachs and John V. Torbert, Jr. (intr. by E. K. Marshall, Jr.). Dept. of Medicine, Johns Hopkins Univ., Baltimore, Md.

Two Negro patients were observed to develop hemolytic anemia while receiving nitrofurantoin for treatment of urinary tract infections. One of these patients was available for further study. It was found that his erythrocytes contained less than the normal concentration of glutathione. Upon incubation of his blood with acetylphenylhydrazine, there was a precipitous fall in erythrocyte glutathione content. These changes are like those shown by Beutler et al. to be characteristic of the intrinsic red cell defeat of those individuals uniquely susceptible to the hemolytic action of certain drugs such as primaquine. Further study demonstrated a striking fall in erythrocyte glutathione upon incubation of 1.0 ml of patient's whole blood with 0.1 mg nitrofurantoin whereas no essential change was observed in the glutathione of normal cells with as much as 1.0 mg/ml. Blood was obtained from 4 other Negro subjects whose erythrocytes had previously been determined to be of the primaquine-sensitive type by incubation with acetylphenylhydrazine. A marked reduction of erythrocyte glutathione was observed in each of the 4 when 1 ml of blood was incubated with 0.1 mg nitrofurantoin. It thus seems probable that nitrofurantoin may induce hemolytic anemia in the same subjects who could be expected to have a hemolytic reaction during administration of primaquine, and by the same mechanism. In a random survey of 140 Negroes, 3 primaquine-sensitive subjects were found, an incidence of about 2%.

1337. URINARY EPINEPHRINE AND NOREPINEPHRINE EXCRETION DURING ACUTE ALCOHOL INTOXICATION IN DOGS. Gerda I. Klingman and McC. Goodall (intr. by Frederick Bernheim). Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.

Intact and bilaterally adrenalectomized dogs were subjected to alcohol intoxication. Urine from these dogs was collected at 3-hr intervals over a 24-hr period and bioassayed for epinephrine and norepinephrine (von Euler and Hellner. Acta physiol. scandinav. 22:161, 1951). Acute sublethal alcohol intoxication (8 ml/kg as a 25% solution administered by stomach tube) in intact unanesthetized dogs is accompanied by an increased excretion of both epinephrine and norepinephrine. Epinephrine excretion is increased up to 20 times above control values during the first 9 hr of the intoxication after which it gradually declines. Norepinephrine excretion is increased 6-20 times above control values and this occurs in two peaks,

between 6 and 9 hr and between 21 and 24 hr after the alcohol administration. When the same dose of alcohol is administered to bilaterally adrenalectomized dogs (maintained on hydrocortisone and desoxycorticosterone acetate) the epinephrine excretion is not significantly elevated above control values; however, the norepinephrine excretion is elevated, although to a somewhat lesser degree than in the intact dogs. Furthermore, 24 hr after alcohol administration the epinephrine content of the adrenal gland was determined and found to be 50% of normal; the norepinephrine content was unaltered. (Supported by Natl. Insts. of Health Grant H-2567.)

1338. KINETICS OF CULLULAR ACTION OF INJECTED BICARBONATE IN CEREBRAL CORTEX. Alan Koch* and Dixon M. Woodbury. Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

Evidence has previously been presented that the extracellular compartment of brain is composed of two phases one, compromising about 4% of total brain weight, allows the entry of both chloride and sulfate ions, whereas the other, comprising about 21% of total brain weight, allows the entry only of chloride ion and is presumed to represent glial tissue. The rate constants for entry into these two phases are 9.23×10^{-3} and 5.02×10^{-4} min, respectively. It has been postulated that cellular water is in equilibrium with the rapidly equilibrating phase of the extracellular compartment. In order to test this hypothesis, the kinetics of the increase in brain excitability resulting from the injection of large doses of bicarbonate ion have been determined. It was found that the change in excitability paralleled the change in the sulfate space and that the maximum increase in excitability occurred at a time when virtually no bicarbonate ion had entered the glial phase. Inasmuch as the concentration of bicarbonate differed in the two phases of the extracellular compartment at the early times, a Donnan equilibrium was induced between the two phases and the concentration of all electrolytes in the glial phase was thereby modified. The implications of this Donnan equilibrium and the methods of calculation of solute concentrations in the fluid compartments of brain under these conditions will be discussed. (Supported in part by Contract No. 18(600)-921. USAF, and Fellowship No. HF-5630-C, Natl. Insts. of Health. PHS.)

1339. METHOD FOR MICRODETERMINATION OF ALLYL ALCOHOL AND ESTIMATION OF CIRCULATING BLOOD LEVELS IN THE RAT. J. K. Kodama,* A. J. Valerga* and C. H. Hine. Dept. of Pharmacology and Exptl. Therapeutics, Univ. of California School of Medicine, San Francisco.

A sensitive method was devised for the determination of microgram quantities of allyl alcohol in water and rat blood. In principle, the method involves the microdiffusion of allyl alcohol into a solution of potassium dichromate in sulfuric acid; the excess dichromate is then reacted with potassium iodide, liberating iodine. The blue color resulting from reaction of iodine with starch is measured spectrophotometrically. A linear relationship was found for concentrations ranging from 1 to 8 μg/0.1 ml of alcohol in either water or rat blood. Mean portal venous blood levels of allyl alcohol in rats given 120 mg/kg perorally (approximate LD_{50}) ranged between 9 and 15 μg/ml for from 15 to 120 min. Mean vena caval blood levels after 30 mg/kg i.v. declined rapidly from 23 μg/ml at 5 min to 4 at 15 min, 3 at 30 min, 0.4 at 60 min and 0 at 120 min. Continuous intravenous infusion at graded dose levels indicated that the rat was capable of metabolizing allyl alcohol at a rate not exceeding 23 mg/kg/hr. All rats showed definite gross signs of toxicity. The distinctive histopathologic finding, periportal hepatic necrosis, was seen consistently. (Supported in part by a grant from the Shell Development Co., Emeryville, Calif.)

1340. HISTOCHEMICAL DEMONSTRATION OF ANTICHOLINESTERASE ACTION AT SELECTIVE SITES IN VIVO. George B. Koelle. Dept. of Physiology and Pharmacology, Grad. School of Medicine, Univ. of Pennsylvania, Philadelphia.

N,N'-bis (2-diethylaminoethyl) oxamide bis-2-Cl-benzyl Cl (WIN 8077, ambenonium Cl) and its 2-methoxy analog (WIN 8078) have been shown to produce characteristic anti-ChE effects at doses far below those causing measurable inhibition of AChE (Lands et al., 1955; Karczmar, 1957). The bis-quaternary structures and reversibility of these agents suggest they might cause ordinarily undetectable ACh - inhibition at strategic sites external to relatively impermeable membranes. A test of this possibility was provided by the observation that physostigmine, by combining with AChE, protects cats against the irreversible lipoid-soluble inhibitor, DFP (Koster, 1946; Koelle, 1946). Anesthetized (Na pentobarbital), atropinized cats were injected i.v. with 0.3 to 300 μg WIN 8077/kg, or equivalent doses of WIN 8078; 5 min later they received 3.7 mg DFP/kg, i.v.; after 30-40 min, they were killed by i.v. air, and the stellate and ciliary ganglia and a portion of intercostal muscle were removed. Frozen sections were cut immediately, placed on slides and kept for 30 min in each of 3 changes of storage solution; slides were incubated in acetylthiocholine- Cu glycinate medium for 5, 10, 20, 40 and 80 min and developed with $(NH_4)_2S$. Sections of ganglia from control cats which had received only DFP were completely blank; motor endplates were stained faintly. Ganglia of cats which had received 3 μg (1/2 after 0.3 μg). WIN 8077 or 30 μg WIN 8078/kg, or more, were stained consistently, with increasing intensity at higher doses and longer incubation periods. Staining was most marked in the protoplasmic tracts of stellate ganglia and immediately surrounding ganglion cells of ciliary ganglia. Cytoplasm of ciliary ganglion cells, which stains intensely in untreated controls, remained blank. (Supported by Public Health Service Grant No. B-282 (C-3 and C-4).)

1341. ANTAGONISM TO CHOLINESTERASE INHIBITORS BY BENZOQUINONIUM. Gilbert Y. Koff* and J. M. Coon. Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.

The neuromuscular blocking agent benzoquinonium, which also possesses anticholinesterase activity, has been shown, if combined with atropine, to protect mice poisoned with TEPP. To investigate whether benzoquinonium antagonizes other cholinesterase inhibitors it was administered intraperitoneally to male mice at several intervals prior to doses of various inhibitors which killed 80-100% of the controls. Benzoquinonium was almost completely ineffective against the agents which strongly affect brain cholinesterase in vivo e.g. parathion, DFP. The compound moderately antagonized those agents which are less effective in their in vivo inhibition of brain cholinesterase such as TEPP and EPN, while it best antagonized OMPA, bis-(dimethylamido) fluorophosphate (BFP), and the symmetrical and unsymmetrical diethyl bis(dimethylamido) pyrophosphates (6400, 6515) which exert little in vivo inhibition of brain cholinesterase. Benzoquinonium also moderately antagonized the reversible inhibitors

physostigmine and neostigmine. Treatment by atropine or scopolamine combined with benzoquinonium increased the LD_{50} of OMPA approximately 10-fold. The otherwise irreversible effect of TEPP on the isolated rabbit ileum was easily reversed by washing if either benzoquinonium or eserine was added to the bath previously. In preliminary tests benzoquinonium did not affect the brain cholinesterase of rats. When dog erythrocytes were incubated with 10^{-4} M benzoquinonium and 10^{-4} M DFP and dialyzed, the cholinesterase inhibition was 16% compared to 91% in a DFP control. It is believed that benzoquinonium, by forming a reversible enzyme complex at peripheral sites, inhibits the formation of an irreversible complex and thus affords protection against those anticholinesterases which do not strongly affect brain cholinesterase in vivo.

1342. INFLUENCE OF BIOFLAVONOIDS IN A SMALL CONTROLLED SERIES OF RESPIRATORY INFECTIONS IN CHILDREN. Robert L. Korn and William T. K. Bryan (intr. by D. M. Green). St. Vincent's German Orphan Home and Dept. of Otolaryngology, Washington Univ. School of Medicine, St. Louis, Mo.

A soluble lemon bioflavonoid fraction was administered orally to 17 children suffering from upper respiratory infections. Half the group received ascorbic acid in addition to the bioflavonoid. Another 16 children were treated with either ascorbic acid alone or a placebo. Clinical diagnoses were based on objective findings and were correlated with cytological observations of smears taken from the nasal passages at the beginning, during and at the termination of treatment. Original diagnosis, follow-up observations and cytological diagnoses were kept segregated until completion of each individual case. Composition of the various medications were withheld from the clinical observers until completion of the entire study. Cure rate in the nonbioflavonoid-treated group was 38%, comparable with the rate previously reported in placebo-treated subjects (Beecher. J. A. M. A. 159:1602, 1955). In contrast, the cure rate in the bioflavonoid-treated group was 65 per cent. Follow-up an average of 1 wk later showed no essential change over the initial result. Ascorbic acid failed to show any beneficial influence either when given alone or when added to the bioflavonoid. Though this preliminary study represents a small series, the extent of the cure rate in the bioflavonoid-treated group (about double the control value) suggests that the influence of the medication on the disease process was real and not a result of chance variation.

1343. CHOLINESTERASE INHIBITORS AND SPONTANEOUS ACTIVITY OF THE ILIOFIBULARIS MUSCLE OF THE FROG. Charles P. Kraatz. Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.

Exposure of the isolated iliofibularis muscle of the frog to cholinesterase inhibitors results in repeated spontaneous contractions, which may persist for periods of 12 hr. Tetraethylpyrophosphate treatment (10^{-6}) results in activity of both contractural and twitch systems, while other inhibitors evoke principally twitch activity. Contractural tensions developing spontaneously vary around 2 gm, while twitch tensions are often of the order of 6-8 gm. Exposure of the muscle to tubocurarine (10^{-6}) during or after the period of treatment with tetraethylpyrophosphate eliminates activity, but contractions appear after removal of the curare in the presence or absence of the cholinesterase inhibitor.

1344. URIC ACID STUDIES IN THE CHICK EMBRYO AND CHICK. Irwin H. Krakoff,* David A. Karnofsky, M. Earl Balis* and Corinne Lacon.* Sloan-Kettering Inst., New York City.

Uric acid production in the chick embryo and growing chick has been measured as a possible system for the study of antimetabolities interfering with purine synthesis. Determinations of total uric acid content of fertilized eggs at various stages of incubation revealed the appearance of small amounts of uric acid at 4 days' incubation. The uric acid content increased rapidly and progressively from the 8th day of incubation until 21 days after hatching, after which it tended to level off. The rapid accumulation appeared to be associated with the period of most rapid synthesis of DNA in the embryonal and early posthatching periods and may be related to nucleic acid synthesis. Glycine has been reported as an "essential" amino acid in the chick. Because of its importance in uric acid synthesis, uric acid production was studied in growing chicks on normal (3%) and low glycine (0.3%) diets. Amounts of uric acid produced were similar in each group; it could be shown that the uric acid produced in the glycine-deficient group was greatly in excess of the amount which could have been derived from dietary glycine. C^{14}-labeled glycine was administered to each group. In corporation into uric acid was high and similar in both groups. Free gylcine was also similar in both groups. C^{14}-labeled arginine, in contrast, was not found in uric acid. These observations indicate that the chick uses glycine, as do other systems, for uric acid synthesis, and is capable of synthesizing its own glycine. Introduction of amethopterin and azaserine into the developing chick embryo has produced impairment of uric acid production.

1345. EFFECTS OF GLYCYRRHIZIN ON THE PITUITARY-ADRENAL SYSTEM OF RATS. Shirley D. Kraus,* H. A. Fonrose* and Walter M. Booker. Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.

In a previous report (Draus and Kurtz. Fed. Proc. 15:112, 1956), it was indicated that the desoxycorticosterone-mimetic action of glycyrrhizin (licorice extract) could be demonstrated in rats when using a minerosteroid assay method. To determine whether the pharmacological action of glycyrrhizin further resembles that of desoxycorticosterone, the response of the pituitary-adrenal system of glycyrrhizin fed rats was studied in the following respects: 1) the depletion of adrenal ascorbic acid in response to histamine stress; 2) the mobilization of glucose in response to histamine stress, and 3) the rate of survival during cold stress. There is evidence that glycyrrhizin exerts an inhibitory effect upon the pituitary-adrenal system.

1346. CARDIOVASCULAR REACTIVITY OF RECENTLY NEPHRECTOMIZED DOGS RECEIVING PERITONEAL LAVAGE. E. M. Krieger (intr. by R. P. Ahlquist). Depts. of Physiology and Pharmacology, Med. College of Georgia, Augusta.

Some hypotheses explain experimental hypertension (renal and renoprival) as being due to a failure of a normal kidney function (unknown) that maintains blood pressure at its normal level. One of the results of this failure could be an increase in the reactivity of the heart and blood vessels. Therefore, several experiments were planned to study early changes in cardiovascular reactivity in bilateral nephrectomized dogs receiving peritoneal lavage. The blood pressure, total peripheral resistance and flow (ml/sec/m^2) were measured by the pulse pressure method (optical

manometer in the carotid artery). Responses to epinephrine, levarterenol, hypertensin (angiotonin), nicotine or 1,1-dimethyl-4-phenylpiperazinium iodide, and stimulation of the central end of the vagus nerve were recorded before and after bilateral nephrectomy. Each dog served as its own control. In the first series the bilateral nephrectomy was done in 2 stages, approximately 1 wk apart. Peritoneal dialysis was initiated 20 hr after nephrectomy. When the final reactivity was determined (48 hr) the changes from the control values were as follows (5 dogs): serum Na: 146→146.5 mEq; serum K: 3.6→6.0 mEq; blood NPN: 33.2→140 mg%; blood volume: no greater than 12% change. Data will be presented analyzing the changes in blood pressure, resistance and flow. Current studies on reactivity are being performed on bilateral nephrectomized dogs using ganglionic blocking agents (mecamylamine), sham operation and longer periods of time after nephrectomy. (Supported by grants from the Natl. Heart Inst. and American Heart Assoc.)

1347. EMETIC EFFECTS OF NICOTINE AND LOBELINE. R. J. Laffan* and H. L. Borison. Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

The doses of 1.5 mg/kg nicotine bitartrate and 0.5 mg/kg lobeline sulfate were uniformly effective, in both cats and dogs, in evoking emesis within 15 min after intramuscular injection. In 4 cats, acute spinal cord transection at T-1 combined with bilateral mid-cervical vagotomy abolished the emetic response to these drugs, although other emetic agents still produced vomiting. On the other hand, when transthoracic vagotomy was combined with low cervical spinal cord transection in 2 chronic cats, nocotine and lobeline remained effective. Furthermore, the following chronic procedures, whether performed alone or in combination with bilateral mid-cervical vagotomy, failed to block the vomiting: a) decerebration b) spinal cord transection at T-4; c) bilateral dorsal rhizotomy of segments T-1 through T-10. Five chronic cats, which were refractory to the emetic action of apomorphine or deslanoside as a result of medullary emetic chemoreceptor trigger zone (CT zone) ablation, exhibited inconsistent emetic responsiveness to nicotine and lobeline. However, all of 10 dogs subjected to CT zone ablation were consistently refractory to nicotine. Tetraethylammonium as well as hexamethonium was effective in blocking the vomiting to nicotine and lobeline, and a dose-response relationship was established for these antagonists. By contrast, atropine and chlorpromazine were ineffective in preventing nicotine- and lobeline-induced emesis. (Supported by Grant No. B-491, Natl. Insts. of Health, PHS.)

1348. AGGREGATION, AMPHETAMINE, AND "TRANQUILLIZERS." Louis Lasagna and William P. McCann.* Div. of Clin. Pharmacology, Dept. of Medicine, Johns Hopkins Med. School, Baltimore, Md.

Gunn and Gurd, and Chance, described some years ago the increased toxicity of parenteral amphetamine for mice grouped together or confined in a limited area. We have confirmed these observations and investigated the ability of certain sedatives and "tranquillizers" to antagonize this increased toxicity. Pentobarbital (in doses of 10-60 mg/kg) proved essentially ineffective in protecting grouped or individual amphetamine-treated mice. Phenobarbital was capable of raising the LD_{50} of amphetamine for grouped mice to the level seen in individual amphetaminized control mice, but high doses (150 mg/kg) of phenobarbital were required for this, and the mice exhibited prolonged sedation and ataxia. Chlorpromazine (in doses of 5 mg/kg) had a similar protective effect in grouped mice and did not cause prolonged after-effects. Neither phenobarbital nor chlorpromazine produced significant change in the LD_{50} of amphetamine in individual mice, in contrast to the marked protective activity of these drugs in grouped mice. Promazine was compared with chlorpromazine in grouped mice and appeared less potent than the latter drug in preventing death from amphetamine. Reserpine (in doses of 1-5 mg/kg) conferred protection upon amphetaminized grouped mice similar to that observed with chlorpromazine. (Supported by Public Health Service Grant B865-C, and by Eli Lilly and Co.)

1349. EFFECTS OF 1-(3,4-DIMETHYLPHENYL)-1-DIMETHYLAMINO-4-PHENYL-BUTAN-HYDROCHLORIDE ON CARDIOVASCULAR HEMODYNAMICS. Norman Lasker,* Gerald A. Kien* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

In a study of a series of compounds shown to have smooth muscle relaxant properties, a marked increase in coronary blood flow was noted following the administration of 1-(3,4 Dimethoxyphenyl)-1-dimethylamino-4-phenyl-butan-hydrochloride (SP 281). Further studies were then undertaken on the cardiovascular effects of SO 281 making use of the open chest pentobarbitalized dog in which blood pressure, cardiac output, coronary blood flow, cardiac work, cardiac oxygen utilization and electrocardiographic changes were measured. A logarithmically progressive series of doses, contained in 0.2 ml of 0.9% saline, was injected into the circumflex branch of the left coronary artery over a 10-sec period. Equivalent doses of papaverine hydrochloride were injected in a similar manner and the dose response curves compared. SP 281 was observed to have an effect on coronary blood flow of similar magnitude but of greater duration than that of papaverine hydrochloride. SP 281 produced a slight decrease in cardiac work and cardiac oxygen utilization. This decrease in cardiac work was the result of a reduced stroke volume, blood pressure and heart rate remaining constant. Frequently, an improved electrocardiogram was observed following the injection of SP 281. It would appear that the observed increase in coronary blood flow was the result of either myocardial depression or a direct relaxant effect on the coronary vasculature. (Supported in part by a research grant from the PHS.)

1350. EFFECTS OF MEPROBAMATE ON FEAR AND PALMAR SWEATING. Victor G. Laties (intr. by L. Lasagna). Div. of Clin. Pharmacology, Dept. of Medicine, Johns Hopkins Med. School, Baltimore, Md.

The effects of meprobamate on human subjects in a fear-provoking situation were assessed. Twenty subjects (10 male, 10 female) were given either placebos or 1600 mg of the drug in 4 equal doses 11, 7, 3 and 1 hr. before a determination of palmar sweating. A packet of anhydrous silica gel was held for a 15-min period and the weight increment was taken as the measure. Another determination of palmar sweating was made approximately 45 min later; during the latter 15-min period subjects rode on a ferris wheel. Loop-by-loop self ratings on an 11-point fear-relaxation scale were made during the ride. At the end of the ride, subjects made a

retrospective fear rating and checked a list of emotional symptoms felt during the ride. All subjects went through this procedure on 2 days, with drug order counterbalanced. Under placebo, mean palmar sweat readings were 282 mg before the ride, 388 mg for the period including the ride; under meprobamate, the readings were 238 mg and 337 mg, respectively. Neither the drug effect nor the drug-ride interaction was statistically significant; the ride itself was associated with a significant increase in sweating. Drug treatment did not alter the loop-by-loop and retrospective fear ratings, but subjects reported afterward that they had significantly fewer emotional symptoms on the wheel when on meprobamate. (Supported in part by Public Health Service Grant B865-C.)

1351. METABOLIC DEGRADATION OF PROPOXYPHENE, A NEW ANALGESIC. Henry M. Lee, Earl G. Scott* and Albert Pohland.* Lilly Research Labs., Indianapolis, Ind.

Alpha-dl-4-dimethylamine-1,2-diphenyl-3-methyl-2-propionoxybutane hydrochloride, or Propoxyphene, is a new therapeutic agent capable of elevating the threshold of pain in animals and man. (Gruber et al. Arch. intern. pharmacodynamine 104:156, 1955, and Robbins. J. Am. Pharm. Assoc. 44:497, 1955). The metabolic degradation of this substance by rat liver slices and the excretion of metabolic products in human urine have been studied. A sample of the drug, labeled with C^{14} in the N-methyl group, with a specific activity of 2.0 $\mu c/\mu M$ was made available. When approximately 0.68 μM of the labeled drug was incubated 30 min with 0.5 gm of rat liver slices, 12.3% of the radioactivity initially added to the incubates was recovered as $C^{14}O_2$. This indicates de-methylation as one of the pathways of metabolism. Since removal of one or both methyl groups should yield a compound capable of forming a derivative with dinitrofluorobenzene, evidence for this derivative has been sought in extracts of liver incubates of Propoxyphene and has been obtained. The urines of 6 volunteers receiving the unlabeled drug have yielded a crystalline metabolite isolated as the DNP derivative. Elemental and physical analysis are consistent with the structure of N-des methyl Propoxyphene.

1352. INOTROPIC ACTIONS OF CERTAIN QUATERNARY AMMONIUM COMPOUNDS. Woo Choo Lee* and F. E. Shideman. Dept. of Pharmacology and Toxicology and Cardiopulmonary Research Lab., Univ. of Wisconsin Med. School, Madison.

The inotropic effects of tetramethyl-, tetraethyl-, tetra-n-propyl-, tetra-n-butyl-, trimethylphenyl-, trimethylbenzyl-, and trimethyl-β-phenethyl-ammonium were studied on the isolated papillary muscle of the cat and in the heart-lung preparation of the dog. Papillary muscles were prepared, as described by Cattell and Gold, and placed in a bath containing Tyrode's solution aerated with a mixture of 95% O_2 and 5% CO_2. The muscles were stimulated continuously with a square wave impulse at the rate of 1 per second and the isotonic contractile amplitude recorded. In experiments with the heart-lung preparation, the procedure of Starling was employed. Failure in both preparations was induced by pentobarbital. Tetramethylammonium ($2x10^{-2}$ M) and trimethylbenzylammonium ($3.3x10^{-2}$ M) decreased the contractile amplitude of the papillary muscle and both compounds produced failure in the heart-lung preparation. All of the other analogs exhibited a positive inotropic action in both fresh and hypodynamic papillary muscle and heart-lung preparations. The minimal effective concentration of tetraethylammonium in the case of the papillary muscle was $2x10^{-4}$ M; the hypodynamic heart-lung preparation responded to 1 mM (total dose). Trimethylphenylammonium was approximately 4 times more potent in both hypodynamic papillary muscle and the heart-lung preparations. The effect of a single dose of trimethylphenylammonium persisted for more than 2 hr in contrast to a much shorter duration of action of tetraethylammonium. The tetrabutyl derivative, at concentrations above $5x10^{-4}$ M, produced a biphasic response in both preparations, first a positive, then a negative inotropic action. The remaining compounds were qualitatively similar in action to tetraethylammonium but, in general, less potent.

1353. PATHOGENESIS OF CARDIOVASCULAR NECROSIS INDUCED BY NEPHRECTOMY. David Lehr, Isabel Wajda* and Marilyn Krukowski.* Dept. of Physiology and Pharmacology, New York Med. College-Flower and Fifth Ave. Hosps., New York City.

It was demonstrated in earlier studies that severe cardiovascular and smooth muscle necrosis is readily induced in the albino rat by means of standard renal injury (Lehr et al. Bull. New York M. Coll. 15:130, 1952) and that this muscular necrosis is a consequence of autointoxication with parathyroid hormone (Lehr and Martin. Proc. Soc. Exper. Biol. & Med. In press). Since the parathyroid glands were apparently stimulated by phosphate retention and increased mineralocorticoid production resulting from severe impairment of renal excretory function, it was reasoned that outright removal of both kidneys, if survived long enough, should induce similar cardiovascular injury, based on the same mechanism. It was found that albino rats survived bilateral nephrectomy usually for 3-4 days, and that the majority manifested extensive and grossly visible cardiovascular necrosis. The incidence and severity of these lesions were substantially enhanced by administration of parathyroid extract on the 1st and 2nd day after nephrectomy. A similar aggrevating effect was achieved in nephrectomized as well as nephrectomized-adrenalectomized rats by injection of desocycorticosterone. However, when rats were deprived of their parathyroid glands prior to nephrectomy, they failed to develop any cardiovascular necrosis. These results indicate that autointoxication with parathyroid hormone can be induced by removal of the kidneys as readily as by impairment of renal function. Hence they prove conclusively that a renal mechanism or "poison" is not involved in the development of this experimental muscular necrosis. (Supported by Research Grant No. H-890 (C5) from the Natl. Heart Inst., PHS.)

1354. POTASSIUM TRANSFER FROM PLASMA TO BILE IN THE ISOLATED PERFUSED RAT LIVER. G. F. Leong,* R. J. Holloway* and R. W. Brauer. U.S. Naval Radiological Defense Lab., San Francisco, Calif.

Previous investigations by the authors using radioactive Na^{22}, K^{42}, Cl^{36}, and $P^{32}O_4$ to study their transfer from plasma to bile in the isolated perfused rat liver have suggested that a passive transfer is involved for the cations. This process was further studied with the double perfusion technique as described by this laboratory which allowed the liver to be instantaneously transferred from an inert perfusate (100% rat plasma) to an identical perfusate containing labeled material. A Geiger-Muller tube was placed directly over the perfused organ in such fashion that liver activity was continuously recorded. Experiments in which the K concentration in the labeled perfusate

was either low, 1-3 mEq/l. or high, 15-20 mEq/l. were conducted to study the effect of varying the concentration gradient from plasma to bile. The results of these experiments which may help elucidate the process involved in the transfer of K ions from plasma to bile are: a) total bile K is significantly higher than plasma K, approximately 20%; b) this difference is independent of the K concentration of the perfusate: c) the bile and plasma specific activities are identical under the various experimental conditions described, and d) bile K is always directly derived from plasma and apparently the transfer does not involve exchange with intracellular potassium ions. The data suggest that plasma K is able to freely diffuse into bile.

1355. ATRIAL FIBRILLATION AND HYPOPOTASSEMIA. Phillip E. Leveque (intr. by R. P. Ahlquist). Dept. of Pharmacology, Med. College of Georgia, Augusta.

Insulin causes a transfer of glucose into the body cells. This transfer is accompanied by potassium ions. In the intact anesthetized dog the intravenous administration of insulin in a dosage of 0.25-1.0 unit per kilogram resulted in a depression of serum potassium to levels as low as 60% of "normal" within about 30 min. This effect persisted for approximately 2 hr. When the serum potassium level was depressed to about 75% of "normal," atrial asystole, fibrillation and flutter could be provoked by the intravenous administration of small doses of acetylcholine which did not produce such an effect during the control period. The period of predisposition to these arrhythmias persisted until the serum potassium level began to rise from the low levels observed. After about a 10% rise from the lowest level, the atria again became resistant to the effects of the intravenous acetylcholine. Infusion of acetylcholine during the falling phase or period of maximum depression of serum potassium prolonged these arrhythmias as long as the infusion was continued. Intravenous injection of potassium chloride would arrest these arrhythmias during acetylcholine infusion and serum levels above about 75% of "normal" prevented the induction or reinduction of these arrhythmias. (Supported by Grant H-240 from Natl. Heart Inst., PHS.)

1356. PHYSIOLOGIC DISPOSITION OF HEXAMETHONIUM AND CHEMICALLY RELATED COMPOUNDS. Ruth R. Levine and Byron B. Clark. Dept. of Pharmacology, Tufts Univ. School of Medicine, Boston, Mass.

The fate of hexamethonium (C_6) was studied as part of a more extensive investigation designed to elucidate the basic principles underlying the physiologic disposition of onium compounds and the relation of physiologic disposition to pharmacologic activity. Hexamethonium is not metabolized in the intact animal in periods up to 18 hr as evidenced by the recovery of 95-103% of the total dose administered to mice. The amount of C_6 absorbed, 33% in 3 hr, from in vivo intestinal loops in the rat is greater than that of Benzomethamine or other onium compounds studied (J. Pharmacol. & Exper. Therap. 114:78, 1955). Also the percentage of C absorbed increases with time although the rate of absorption decreases. The pattern of tissue distribution of C_6 in the rat differs considerably from that of the monoquaternary compounds already investigated (ibid. 114:63, 1955). The latter were found primarily in liver, kidney and, to an even greater extent, in small intestine and could not be detected in blood, muscle or fat. In contrast, C_6 is distributed in blood, muscle and fat; is not concentrated in the liver; and is found in the small intestine to a limited degree in only one-half of the animals studied. This latter fact can be explained by the finding that only 0-3% of the total dose is excreted in the bile. Other analogues of C_6, including the monoquaternary derivative and the sulphonium analogue, were studied in a similar manner and will be discussed.

1357. INHIBITION OF ADRENERGIC DEPRESSOR RESPONSE. B. Levy* and R. P. Ahlquist. Dept. of Pharmacology, Med. College of Georgia, Augusta.

The ability of a number of drugs to inhibit the vasodepressor response to 3 different adrenergic agents in dogs has been determined. Epinephrine, isoproterenol, and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) administered to dogs treated with phenoxygenazmine were the drugs used to produce the vasodepressor responses. DMPP, a ganglionic "stimulant," following phenoxybenzamine produced an initial pressor response followed by a pronounced depressor response. After ephedrine the depressor responses of epinephrine and DMPP were converted to purely pressor responses while the depressor effect of isoproterenol was inhibited. In order to determine if this action of ephedrine was typical of the adrenergic agents in general or true only in the case of ephedrine, the following adrenergic agents were tested: phenylpropanolamine, 2-methylaminoheptane, hexyl-B-cyclohexylethylamine, N-cinnamyl-2-phenylpropylamine, phenylpropylmentylamine, N-ethylephedrine and amphetamine sulfate. None of these compounds was effective in blocking the vasodepressor effects of epinephrine, isoproterenol or DMPP. Studies on the interrelation of these drugs on isolated tissues and on regional blood flow are in progress. (From the Cardiovascular Research and Training Program supported by grants from Natl. Heart Inst., PHS and American Heart Assoc.)

1358. EFFECTS OF ACID-BASE BALANCE ON DIURESIS INDUCED BY ORGANIC AND INORGANIC MERCURIALS. R. I. Levy,* I. M. Weiner* and G. H. Mudge. Dept. of Pharmacology, Johns Hopkins Univ. School of Medicine, Baltimore, Md.

The effects of metabolic acidosis and alkalosis on the chloruresis induced by organic mercurials have been generally attributed to changes in the load of filtered chloride presented for tubular reabsorption. Studies on the alkalosis of potassium depletion suggested an additional mechanism, namely, that the interaction between the organic mercurial and some essential renal constituent might be pH dependent. By analogy with the studies of Benesch and Benesch, it was suggested that such a pH dependent reaction might involve the destruction of the organic mercurial with the liberation of inorganic mercury. To test this hypothesis comparison was made between the diuretic action of mercuric chloride and meralluride in both alkalotic and acidotic dogs. While alkalosis completely inhibits chloruresis due to meralluride, it has little inhibitory effect on chloruresis due to mercuric chloride. This marked difference cannot be explained on the basis of filtered chloride load and is consistent with the hypothesis that the organic mercurials act by liberation of inorganic mercury.

1359. EFFECT OF BORIC ACID AND SODIUM METABORATE ON OXYGEN UPTAKE OF BRAIN TISSUES. Alfred S. C. Ling,* Edward B. Truitt, Jr. and John C. Krantz, Jr. Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore.

Boric acid was shown to exercise a negligible inhibitory effect on the oxygen uptake of excised rat

cerebral tissue when substrate is present. In the presence of no substrate, boric acid and sodium metaborate markedly depress oxygen utilization under the same conditions. The degree of inhibition by boric acid appears to be about the same for the 4 areas of the brain, namely, the cerebrum, cerebellum, medulla oblongata and hypothalamus-thalamus.

1360. METHITURAL CARDIAC ARRHYTHMIAS IN DOGS AS INFLUENCED BY SYMPATHOMIMETIC AMINES. L. H. Lonergan* and C. M. Gruber. Dept. of Pharmacology and Exptl. Therapeutics, College of Med. Evangelists, School of Medicine, Loma Linda, Calif.

Previous investigations have demonstrated a high incidence of cardiac arrhythmias in dogs under intravenous Methitural anesthesia. This study concerns the effect of various sympathomimetic drugs on this Methitural arrhythmia. Of 25 sumpathomimetic amines tested by rapid intravenous injection in the dog, Isoproterenol Hydrochloride (Aludrine Hydrochloride and Isuprel Hydrochloride) and Isoproterenol Sulfate (Norisodrine Sulfate), in the doses used, restored the heart to normal rhythm in every instance. Adrenaline chloride, L-Norepinephrine, L-epinephrine and orthoxine were but slightly less efficient, bringing about normal cardiac rhythm in most trials. With all other sympathomimetic amines tested, the results in establishing normal rhythm were poor and unpredictable. In our experiments, the restoration to normal rhythm appears to be due, not to any blood pressure change, but primarily to a direct action of the sympathomimetic amine on the myocardium.

1361. IDENTIFICATION OF BARBITURATES FOR FORENSIC MEDICAL PURPOSES BY USE OF X-RAY DIFFRACTION. Ted A. Loomis, James E. Fox* and Carolyn S. Spaeth.* Dept. of Pharmacology and State Toxicology Lab., Univ. of Washington, Seattle.

Barbiturates and mixtures of barbiturates can be specifically and absolutely identified in concentrations commonly encountered in Forensic Medicine by the X-ray diffraction procedure. The method used in this laboratory involved chloroform and water extraction of the barbiturate and vacuum sublimation of the extract. Recovery of added barbiturate in control preparations was accurate to 0.1 mg %. The sublimated crystals, in acid form, were transferred to the diffraction capillary tube and the diffraction pattern recorded on an automatic microdensitometer. Standard diffraction patterns of 6 commonly encountered barbiturates were established. Five-tenths milligram of barbiturate in blood, urine or tissue (liver) has been extracted and absolutely identified by this procedure, which has also been successfully used in identifying mixtures of 2 barbiturates in a single sample.

1362. EFFECTS OF VARIOUS DRUGS ON DURATION OF INTRADERMAL ANESTHESIA PRODUCED BY A DENTAL ANESTHETIC SOLUTION. F. P. Luduena. Sterling-Winthrop Research Inst., Rensselaer, N. Y.

Piperoxan, adenosine and hyaluronidase, alone and in combination, greatly reduced the duration of anesthesia when injected into a wheal raised by the intradermal injection of a solution containing 2% procaine HCl, 0.4% propoxycaine HCl and levo-arterenol 1:30,000. The experimental procedure was as follows: 2 wheals were raised on the back of a guinea pig with the dental anesthetic solution (0.25 cc) and 20 min later 1 of the wheals was reinjected with 0.25 or 0.5 cc of the experimental solution and the other with the same volume of saline. Readings (Bullbring and Wajda's technique) were taken every 10 min until the anesthesia disappeared or was greatly reduced. Each test was carried out on 8 or more animals. Duration of anesthesia represented the time from the 2nd injection to the point in the average anesthetic score-time curve at which the score was 50% of maximum. Piperoxan in concentrations of 0.025 and 0.05% reduced the duration to approximately one-third of the control value. Adenosine (0.2%) and hyaluronidase (15 TRU/cc) were considerably less effective. A mixed solution of piperoxan, adenosine and hyaluronidase was approximately twice as active as the piperoxan solution. Piperoxan adrenolysis, inhibiting or reducing the effect of L-arterenol, adenosine vasodilatation and the facilitation of difusion induced by hyaluronidase explain the effects described.

1363. FIBROSARCOMAS INDUCED BY MULTIPLE SUBCUTANEOUS INJECTIONS OF CARBOXYMETHYLCELLULOSE (CMC), POLYVINYLPYRROLIDONE (PVP), AND POLYOXYETHYLENE SORBITAN MONOSTEARATE (TWEEN 60). Lehman M. Lusky and Arthur A. Nelson (intr. by Harold C. Hodge). Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

Groups of rats each received 1 cc weekly subcutaneous injections of one of the following aqueous solutions: CMC, 2%; PVP, 6%; Tween 60, 6%; dextran 6%; or sodium chloride, 0.9%. Each group of 30 rats was made up of 20 Osborne-Mendel evenly divided by sex, and 10 Bethesda Black males. After 73 wk the total number of rats with injection site tumors was CMC, 13 or 43%; PVP, 13 or 43%; Tween 60, 5 or 17%; dextran, none; sodium chloride, none. By rat strain and sex, the total number of injection site tumors was Osborne-Mendel male, 9; Osborne-Mendel female, 6; Bethesda Black male, 16. No influence of the injections on spontaneous-type tumor production was seen, except that 1 rat on CMC and 3 on Tween 60 (these 4 did not have injection site tumors) had large intra-abdominal lymphosarcomas; the latter figure is higher than would be expected. Injection of CMC left persisting deposits of it at the injection site; this was not evident with the other materials. Microscopically, the injection site tumors were fibrosarcomas of moderate histological malignancy. Grossly, they ranged in size from 1.5 cm spherical to 10 x 6.5 x 4 cm. Hueper (Proc. Am. A. Cancer Res., 2:120, 1956) has reported PVP to be cancerigenic in rats.

1364. COMPARISON OF GLYCYRRHIZIN AND CORTISONE ON SOME PHYTOTOXIC SERA. David I. Macht. Div. of Pharmacology, Labs. of Sinai Hosp., Inc., Baltimore, Md.

In 1951 the author reported experiments in vitro concerning the detoxification of hydroponic solutions of sera in which Lupinus albus seedlings were grown by minute quantities of cortisone and ACTH. Thus the phytotoxicity of pemphigus and psychotic sera for normally reared seedlings was diminished. Similarly, phytotoxic effects of psoriatic sera on vernalized seedlings were also neutralized (Macht. Arch. internat. pharmacody. 86:363, 1951). Recently experiences with glycyrrhizin have been described indicating a similarity in some respects between it and corticotropic hormones (Hudson et al. New England J. Med. 251:641, 1954; Molhuysen et al. Lancet 2:381, 1950). The writer tested strong solutions of glycyrrhizin in vitro on Lupinus seedlings in solutions of psychotic sera 1% and found that such solutions, 1:5,000

definitely were detoxifying. It was found also in this laboratory that cortisone solutions, 1:2,000,000 and even 1:4,000,000, detoxify cancer blood sera tested on root growth of vernalized Lupinus albus seedlings. However, glycyrrhizin even in concentrated solutions produced no such detoxification.

1365. PARTIAL FRACTIONATION OF PARATHYROID EXTRACT. Delmar J. Mahler,* Donald A. Davis* and A. R. McIntyre. Dept. of Physiology and Pharmacology, Univ. of Nebraska, College of Medicine, Omaha.

Methods permitting the assay of small quantities of various parathyroid extract preparations have been developed. Male mice of uniform age and weight (18-20 gm) are maintained on an adequate diet and allowed water ad lib. Blood inorganic phosphorus and serum calcium concentrations are determined 3 and 24 hr, respectively, following injection. The amounts required for injection expressed in U.S.P. parathyroid equivalents are as little as 0.00075 ml/gm of mouse for the assay of "blood phosphorus activity," and 0.0112 ml/gm of mouse for the assay of "serum calcium activity." An acetic acid extract of acetone dried whole parathyroid was shown to have a "calcium activity" of 4.10 "units" per milligram dry weight and a "phosphorus activity" of 4.35 "units" per milligram dry weight. An ultrafiltrate of this same material contained 12.6 "calcium units" and 10.44 "phosphorus units" per milligram dry weight (a Ca/P ratio of 1.21 whereas with nonfilterable material the Ca/P ratio was 2.21). Comparisons of the Ca/P activity ratios are used to evaluate the relative activities of various parathyroid fractions. The results indicate that parathyroid extract contains more than one active principle having separable quantitative effects upon the serum calcium and blood phosphorus.

1366. COMPARATIVE EFFECTS OF MALEIC HYDRAZIDE AND P-DIMETHYLAMINOAZOBENZENE ON RAT LIVER. W. A. Mannell and H. C. Grice (intr. by F. C. Lu). Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Canada.

Thomson et al. (Biochem. J. 53:460, 1953) fed a carcinogenic diet containing 0.06% p-dimethylaminoazobenzene (DAB) to rats for periods up to 6 mo and found an increase in the total number of cells in the liver, a decrease in average size of the cells and a lower average content of DNA/nucleus. These effects occurred both in livers with obvious tumors and in livers that had not reached this stage. Similar effects were observed with thioacetamide (Laird. Arch. Biochem. 46:119, 1953), a compound that has also been reported as a hepatic carcinogen (Fitzhugh and Nelson. Science 108:626, 1948). Rats were fed a stock diet containing 2% of the sodium salt of maleic hydrazide for periods up to 6 mo. At varying times groups of animals were killed, the liver was removed and weighed, a portion was taken for histological study, a 2nd portion was used for whole tissue analysis and the remainder was used for the isolation of nuclei. The same procedure was carried out on rats receiving 0.06% DAB in their diet. Estimates were obtained for the total number of cells in the liver, the weight of the average cell and the average DNA content per nucleus. The concentration of DNA, RNA, acid-soluble and total lipid phosphorus was determined in the whole tissue sample. No significant differences in any of these parameters were found between control rats and those fed maleic hydrazide. The results for the rats fed DAB confirmed the work of Thomson et al.

1367. OCCURRENCE AND POSSIBLE ROLE OF SEROTONIN IN FASCIOLA HEPATICA. Tag E. Mansour, Argimiro D. Lago* and James L. Hawkins.* Dept. of Pharmacology, Louisiana State Univ. School of Medicine, New Orleans.

Lysergic acid diethylamide and serotonin stimulate the rhythmical activity of the liver fluke Fasciola hepatica (Mansour. Fed. Proc. 15:1480, 1956). Other compounds which have similar effects on this parasite are: tryptamine, bufotenine, 5-hydroxytryptophan, amphetamine, ephedrine, mephentermine and mescaline. Indolamines are much more potent in this respect. Serotonin is the most potent stimulatory amine. Bromo-lysergic acid diethylamide (1×10^{-7} M), harmine (1×10^{-5} M) and yohimbine (1×10^{-4}M) inhibit the rhythmical activity of these parasites. The latter drugs antagonize the stimulatory effects of serotonin. In an attempt to determine whether serotonin is present in the flukes, acetone extracts of the parasites were assayed on sensitized rat uterus preparations (Amin, Crawford and Gaddum. J. Physiol. 126:596, 1954) in the presence of atropine. By the use of this procedure a serotonin-like substance was detected in these extracts (0.06-0.07 μg/gm wet wt). The flukes contain a system catalyzing the biosynthesis of serotonin. Incubation of homogenates of Fasciola with 5-hydroxytryptophan and pyridoxal phosphate for 1 hr resulted in a 100-300 fold increase in the amount of serotonin in the reaction mixture. Based on these observations, the possibility will be discussed that serotonin or a related compound is involved in the transmission of nerve impulses in Fasciola. (Supported by research grants from the PHS and from Swift and Co.)

1368. PHARMACOLOGICAL ACTIVITY OF 2-AMINO-5-CHLOROBENZOXAZOLE (ZOXAZOLAMINE, FLEXIN). II. DURATION OF ACTION. David Fielding Marsh. Div. of Med. Sciences, McNeil Labs., Inc., Philadelphia, Pa.

Since the original investigation (Fed. Proc. 14:366, 1955) of the lissive action of 2-amino-5-chlorobenzoxazole, this activity has been quantitatively determined. The duration of action in man and animals is particularly interesting. The effective duration of flaccidity of a dose midway between that producing loss of the righting reflex in one-half of a group of animals (PD_{50}) and that producing respiratory failure in one-half of another group of animals (LD_{50}) has been determined in the following species (M-mouse, R-rat, H-hamster, D-dog):

	Route	PD_{50}	LD_{50}	Median	Duration
M	I.P.	82	351	216	160'
M	Oral	288	688	478	142'
R	Oral	135	782	458	234'
H	I.P.	72	350	211	67'
H	Oral	221	673	447	155'
D	I.V.	37	117	77	24'

The comparative figures for mephenesin are:

M	I.P.	163	546	355	18'
M	Oral	350	912	631	55'

Additional studies with 1, 2 and 3 times the minimal paralytic dose (82 mg/kg) indicates that the drug disappears in mice at a rate of approximately 1 mg/kg body weight per minute within the dosage limits given. Confirmation of this figure has been established by serial injections of 35 and 50 mg/kg doses at the time a mouse regains his righting reflex; the re-establishment of the loss of the righting reflex for 35 and 50 min periods could be maintained indefinitely. Clinical use of zoxazolamine (at oral doses of 3-10 mg/kg b. wt., depending upon age and severity of condition) for the relief of muscle spasticity and spasms indicates that effective duration of action is approximately 6 hr.

1369. ROLE OF THYMUS GLAND IN PATHOGENESIS OF RENOGENIC ARTERIOSCLEROSIS. Constance R. Martin* and David Lehr. Dept. of Physiology and Pharmacology, New York Med. College, Flower and Fifth Ave. Hosps., New York City.

It was demonstrated that removal of the thymus gland enhances arterial medionecrosis induced by standard renal injury in the adult male rat. Since thymectomy did not affect renogenic arteriosclerosis in adrenalectomized rats maintained on cortisone, the suggestion was made that the thymus gland might influence the hormonal output of the adrenal cortex (Martin and Lehr. J. Pharmacol. & Exper. Therap. 116:41, 1956· Fed. Proc. 15:456, 1956). Other experiments from this laboratory have established that parathyroid hormone autointoxication is the basic mechanism underlying development of the arterial lesions and that mineralocorticoids initiate parathyroid hyperfunction (Lehr and Martin. Proc. Soc. Exper. Biol. & Med. In press). To investigate a possible direct effect of thymectomy upon the action of parathyroid hormone, thymectomized thyroparathyroidectomized and sham-thymectomized thyroparathyroidectomized rats were injected daily with 200 U of parathyroid extract. No significant differences in incidence or severity of arterial lesions were found between the 2 groups. In a 2nd experiment, the possible influence of thymectomy upon the delererious effect of a given amount of mineralocorticoid was studied. Thymectomized adrenalectomized and sham-thymectomized adrenalectomized rats maintained on 3 mg cortisone acetate and 1 mg. desoxycorticosterone acetate daily were subjected to standard renal injury. Again, no difference in incidence or severity of arterial necrosis was observed. The data suggest that the thymus gland does not directly enhance the action of either parathyroid hormone or of mineralocorticoids. The results are interpreted as affording further support for the hypothesis that the thymus gland influences the hormonal output of the adrenal cortex. (Supported by research Grant No. H-890 (C5) from the Natl. Heart Inst., PHS.)

1370. GANGLIONIC BLOCKADE IN UNANESTHETIZED DOGS AND ANESTHETIZED HAMSTERS. R. A. Maxwell,* S. Ross* and A. J. Plummer. Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.

A comparative study of the effects of ganglionic blockade on the blood pressure of unanesthetized dogs indicates that doses which produce complete relaxation of the nictitating membrane, and thus a nearly complete blockade, produce marked systolic and diastolic depression in malignant stages of renal hypertension. In normotensive unanesthetized animals the primary fall is in systolic blood pressure. In normotensive dogs anesthetized with barbiturates, ganglionic blocking agents produce clear-cut falls in both systolic and diastolic pressures. It appears, therefore, that the barbiturate-anesthetized normotensive dog is a better model for the study of antihypertensive agents than the unanesthetized normotensive dog. Under anesthesia the decline in pressure produced by ganglionic blockade is often biphasic with a transient period of recovery occurring after an initial dramatic fall, possibly indicating more than a single point of attack. The effects of ganglionic blockade on the peripheral circulation of the anesthetized hamster demonstrate that a transient reduction of peripheral flow does occur within the first 5-20 min after administration, while the lowered blood pressure, which also occurs early, outlasts the period of reduced flow. The reduction in red cell velocity is coupled with arteriolar vasoconstriction and emptying, which disappear as red cell velocity returns to normal. These effects are most likely secondary to the decline in pressure and not the cause of it, and possibly reflect a drastic removal of blood from the general circulation.

1371. ELECTROCARDIOGRAPHIC STUDY OF THE ACTION OF QUINIDINE IN THE DOG. E. L. McCawley, H. L. H. Dick,* D. V. Voiss* and J. D. Krueger.* Dept. of Pharmacology, Univ. of Oregon Med. School, Portland.

Studies of the actions of quinidine in animals using the electrocardiogram have lagged behind clinical information. Using dogs anesthetized with pentobarbital, quinidine gluconate was administered by intravenous infusion until lethal doses had been given. The most striking action of quinidine observed was a slowing of depolarization. This was reflected in a prolongation of the intrinisicoid QRS time interval. The corrected QT_c indicates that much of quinidine's apparent prolongation of QT time (repolarization) is caused by a slowing of the heart rate. Auricular depolarization is also slowed as, following quinidine administration, the corrected PR_c interval is broadened. These effects are apparent with quinidine doses comparable to those used in patients. Quinidine even at relatively low dosage causes a n increase in size (voltage) of the P, S and T waves. These effects are similar to those provoked by an infusion of potassium chloride. Toxic effects of quinidine, in animals given artificial respiration with oxygen, do not, however, resemble profound hyperkalemia. The broadened QRS due to quinidine can be reduced by infusion of 4 mEq/kg sodium lactate. At no time in the dog did quinidine result in the ventricular tachycardia reported in patients with auricular fibrillation receiving quinidine. Nor was ventricular tachycardia seen when quinidine was administered to dogs fully digitalized with digoxin to the point of partial heart block. It is probable that uncompensated congestive heart failure is required for the ventricular arrhythmias of quinidine. (Supported in part by a grant from the Public Health Service.)

1372. ACTION OF THE HISTAMINE RELEASER 48/80 ON CERTAIN ATARACTIC AGENTS. J. D. McColl and P. Sacra (intr. by K. I. Melville). Research Labs., Frank W. Horner Ltd., Montreal, Canada.

Previous observations have demonstrated that nicotine and metrazole induced convulsions in mice are antagonized by benactyzine and chlorpromazine while meprobamate has no such action. The simultaneous administration of the histamine releaser 48/80 (1 mg/kg, i.p.) with chlorpromazine, benactyzine or meprobamate produced faciliation of the CD 50 of nicotine. The metrazole induced convulsions continued to be antagonized in the presence of 48/80 with

these compounds. Benactyzine, chlorpromazine and meprobomate when administered intravenously in the cat produced a fall of blood pressure. Compound 48/80 (15-50 γ/kg) inhibited this hypotensive response when administered 30 min prior to these agents. To obtain this response it was necessary to administer sufficient 48/80 so that subsequent doses of this compound did not produce an additional fall in blood pressure. When LSD (10 γ/kg) was administered following 48/80 this inhibition of the blood pressure fall was abolished and an essentially normal hypotensive response was again observed. These results were uniformly obtained in intact and vagotomized animals.

1373. ABSENCE OF COMPETITION BETWEEN PAH SYNTHESIS AND TUBULAR TRANSPORT OF PAH. B. J. Mc Intosh,* Peter K. Knoefel and Thomas G. Scharff.* Dept. of Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.

Rabbit renal cortical slices were incubated in Ringer's solution containing PABA and glycine. PAH synthesis and PAH uptake by the slices were measured. PAH synthesis progressed at a constant rate (5.3 micromoles/gm tissue/hr) for the entire experimental period of 3 hr. In other tissue samples, Diodrast in concentrations of 10 micromoles/ml of medium failed to inhibit the rate of synthesis of PAH. Dogs were infused with PAH to saturate the tubular secreting mechanism. When PABA was injected into the dogs, excretion of PAH was increased and the apparent T_m was raised. On the basis of these experiments it is concluded that no competition exists between the system responsible for PAH synthesis and the system responsible for tubular transport of PAH.

1374. EXCRETION OF ZINC-65 IN THE BILE, PANCREATIC JUICE, AND INTESTINAL JUICE OF THE RAT. Robert J. McIsaac (intr. by Douglas S. Riggs). Dept. of Pharmacology, Univ. of Buffalo School of Medicine, Buffalo, N. Y.

The excretion of zinc-65 in the bile, pancreatic juice and intestinal juice of the rat after intravenous injection of the chloride was investigated. Bile and pancreatic juice were collected separately by trying small polyethylene tubes in the respective ducts. Two rats each were killed at 2, 4, 6 and 8 hr after injection, and the contents washed from the intestines with saline. Flow of pancreatic juice was stimulated by the hourly administration of secretin and carbachol. Zinc-65 was detected in pancreatic juice within 2 hr after administration of the radioisotope. The amount excreted varied widely among the rats; the values at 8 hr ranged from 0.34% to 1.76% of the dose administered. Zinc-65 was found in bile within 1 hr after injection and was excreted in significant amounts over an 8-hr period. The amount of radioactive Zn recovered from bile was approximately the same as that recovered from pancreatic juice. Increasing bile flow by administration of sodium dehydrocholate had no effect on the total Zn-65 content of bile. Approximately 3% of the dose was recovered from the contents of the small intestines 8 hr after administration, but only small amounts were found in the contents of the large intestine. No significant difference in the excretion of Zn-65 per gram of duodenum, jejunum and ileum was observed. The results suggest that bile and secretions of the small intestine are as important as the pancreatic juice in the elimination of Zn-65 from rats, particularly after large doses of Zn are administered.

1375. METABOLISM OF GLYCEROL ESTERIFIED WITH PALMITIC AND LACTIC ACID. Herbert McKennis, Jr.,* L. B. Turnbull,* Harvey B. Haag and Paul S. Larson. Dept. of Pharmacology, Med. College of Virginia, Richmond.

The metabolism of an ester preparation from the reaction of equimolar quantities of lactic acid, palmitic acid and glycerine was investigated in the dog and in the rat. Within 30 min after ingestion of the ester prepared from lactic acid-2-C-14 labeled lactate was found in the thoracic duct lymph of the dog. For C-14 determination lactate was oxidized to acetaldehyde and then counted as the dimedon derivative. Following dehydration the latter was counted as 2,2,7,7,9-pentamethyl-4,5-dioxooctahydrozanthene. Expired air from rats intubated with an olive oil solution of the ester labeled with glycerol-1,3-C-14 indicated at 45 hr a total metabolic utilization approximately equal to that of an equimolar quantity of glycerol-1,3-C-14 administered in olive oil.

1376. EFFECT OF CERTAIN CARBONYL COMPOUNDS ON GLYOXALASE ACTIVITY. G. R. McKinney. Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgantown.

An extract of rat liver served as the source of glyoxalase (J. Biol. Chem. 219:605, 1956). The effect of some hydroxy- and ketoaldehydes on the activity of this enzyme extract was determined manometrically by measuring the production of CO_2 from methylglyoxal (I). Of a series of alpha-keto - and alpha-hydroxyaldehydes, the former compounds exhibited a greater degree of reactivity with glyoxalase (i.e. conversion to the corresponding acid) when the drug was incubated with the enzyme in the absence of I, as evidenced by the production of a larger volume of CO_2. However, the hydroxyaldehydes did inhibit the conversion of I to lactic acid, as indicated by the decreased release of CO_2. Typical hydroxyaldehydes which were studied and which inhibited glyoxalase activity by 30-50% were beta-diethylaminolactaldehyde, beta-dimethylaminoethoxylactaldehyde, beta-diisopropylaminolactaldehyde and beta-pyrrolidinolactaldehyde. Concentrations of the compounds, higher or lower than 0.01 M (final), showed more or less inhibition, respectively. Amounts of I varying from 60 to 720 μg, with 0.01 M (final) hydroxyaldehyde concentration, showed proportional degrees of glyoxalase inhibition.

1377. EFFECTS OF LEVALLORPHAN-MEPERIDINE COMBINATIONS ON RESPIRATION IN MAN. Robert Megirian* and Chester W. White, Jr. Dept. of Pharmacology, Boston Univ. School of Medicine and Dept. of Anesthesiology, Boston City Hosp., Boston, Mass.

The effects of various combinations of meperidine and levallorphan were tested in 62 postoperative patients who had received spinal or local anesthesia. Minute ventilation, respiratory rate, pulse and blood pressure were recorded on each patient until fairly constant; an intravenous injection of the chosen drug(s) was then administered and measurements were continued at intervals for 60 min (or longer) after the injection. Meperidine was given alone at a dose of 1.5 mg/kg in 10 patients. Various ratios of levallorphan to this dose of meperidine were then prepared; 10 patients each were selected for ratios 1/120, 1/60, 1/30 and 1/15. Saline and levallorphan controls were also done. While levallorphan or saline alone failed to alter respiratory function, meperidine caused a statistically significant fall below the saline controls with respect to minute ventilation for 40 min (74-79% of controls) and respiratory rate for 20 min (82-91% of controls). No significant

differences in minute ventilation between saline and the ratios 1/60, 1/30 or 1/15 were observed; this was also true of respiratory rate except that the 1/15 ratio produced a significant rise above the saline group. The 1/120 ratio was only partially effective in preventing respiratory depression. In trials on patients who had severe pain the 1/60 ratio was found to be effective without causing significant changes in respiration, blood pressure or pulse.

1378. BIOLOGICAL DISPOSITION OF C^{14}-LABELED LEVORPHAN IN DOGS AND MONKEYS. L. B. Mellett* and L. A. Woods. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

N-Methyl-C^{14}-labeled levorphan was administered subcutaneously in a dose of 2 mg/kg (calcd. as free base). In 1 group of experiments, samples of plasma, urine and feces were obtained at selected intervals. In another series of experiments, CO_2 was collected at hourly intervals from a special exposure chamber for 24 hr and total urine was obtained for the 24-hr period. The results of these studies were as follows: In the dog maximum plasma levels for free levorphan of 110-410 millimicrogm (mμg)/ml were obtained 1/2-1 hr after injection and maximum levels for conjugated levorphan of 560-700 mμg/ml were observed 1-2 hr after administration. Maximum plasma levels of the drug in the monkey were similar quantitatively but appeared more quickly after administration. Ten hours after injection, plasma levels of total drug were less than 45 mμg/ml. The recovery of total levorphan in the urine of the dog ranged from 32 to 39% of the total dose (5.2-7.5%, free; 27-31%, conjugated), while in the monkey the recovery of total drug was 25-51% of the dose (2.4-2.8%, free; 22.6-48.4%, conjugated). Fecal recovery in both species was less than 1% of the dose. The excretion of $C^{14}O_2$ in the monkey was 15-22% of the administered radioactivity but in the dog occurred to a much lesser extent. Total radioactivity in the urine of the monkey was significantly greater than that contributed by free and conjugated levorphan. Evidence for the presence of unchanged levorphan and 2 metabolites in the urine of the monkey has been obtained with paper chromatography.

1379. RADIOCHOLESTEROL DEPOSITION AT THE CORONARY ARTERY OF HYPERLIPOPROTEINEMIC DOGS. Lawrence J. Milch and Norman Weiner.* Dept. of Pharmacology-Biochemistry, School of Aviation Medicine, USAF, Randolph AFB, Tex.

Preliminary studies indicated that if radiocholesterol was isolated from the coronary artery and the aorta of a normal dog 24 hr after a single administration of acetate-2-C^{14}, the ratio of C^{14} activities (coronary artery cholesterol/aortal cholesterol) was significantly different from 1. It was decided to study the cholesterol concentrations of coronary artery and aorta in relation to their cholesterol C^{14} activity ratio by administering 10 mc of I^{131} to each of 15 dogs and then maintain the animals until approximately 50 wk after a plasma pattern of low-density lipoproteins had been established. Twenty-four hr after each dog had been given 0.08 mg/kg of acetate-2-C^{14} (1 mc/mm), it was killed by rapid arterial exsanguination and its aorta and coronary artery excised and carefully freed of adhesive fat and connective tissue. The cholesterol concentrations of the aorta and of the coronary artery were measured and the specific activity of radiocholesterol isolated from each tissue determined. Plasma ultracentrifugal lipoprotein and total cholesterol levels were measured and, on additional aliquots of the plasma, lipoprotein density fractions were isolated ultracentrifugally and their individual cholesterol concentrations determined. The data indicate that after 50 wk of hyperlipoproteinemia cholesterol concentrations of the coronary artery and aorta of the dog are well correlated with the rate of deposition of cholesterol, as indicated by the ratio of C^{14} activities. This deposition rate was further studied in relation to plasma concentrations of various lipid parameters. The details of these findings will be discussed.

1380. PROTECTION BY HUMAN GAMMA GLOBULIN AGAINST PSEUDOMONAS AERUGINOSA INFECTIONS IN MICE. R. Carl Millican, Sanford M. Rosenthal and John D. Rust.* Natl. Insts. of Health, Bethesda, Md.

Cortisone pretreatment (5 daily i.m. injections, 1.25 mg/20 gm mouse) enhances susceptibility of mice to Pseudomonas infections, thereby permitting in vivo studies based on mortality (for details see Antibiotics Annual 1956-57. In press). Inocula of 10^5 to 10^6 organisms (i.p.) produce 80-100% mortality in 2-7 days. Only occasional mortality was observed in control mice (cortisone or bacteria alone). Human gamma globulin preparations (including chromatigraphically purified sample obtained from Dr. Herbert A. Sober) markedly protected infected mice; 320 μg, given parenterally within 3 hr after infection, protected half the animals and 3.2 mg protected 86%. Human plasma, but not mouse plasma, protected infected animals in amounts corresponding to gamma globulin content. The factor in human plasma was stable at 65° (30 min min), but destroyed at 85°. Burned mice exhibited increased susceptibility to Pseudomonas infections similar to cortisone-treated mice. Gamma globulin protected burned animals from this infection. Normal mice, infected with larger inocula (10^7 organisms), die within 24 hr, and 30-80% can be protected by gamma globulin pretreatment. Further studies on the synergistic action of combined gamma globulin plus antibiotic therapy, as well as the therapeutic effect of plasmas of other animal species will be reported.

1381. SEIZURE PATTERNS IN RATS IN RELATION TO AGE AND DEVELOPMENT OF BRAIN CARBONIC ANHYDRASE. J. Gordon Millichap (intr. by Alfred Gilman). Depts. of Pediatrics and Pharmacology, Albert Einstein College of Medicine, New York City.

A constant electroshock stimulus (50 ma for 0.2 sec) was used to induce seizures in rats at 18 age levels from birth to 40 days. Brain carbonic anhydrase was determined by the method of Meldrum and Roughton, and the increase in enzyme activity with age was correlated with the electroshock seizure pattern. In the age period 1-10 days, the seizure pattern was characterized by running movements and squealing. No change in response occurred with currents up to 200 ma. Between 10 and 20 days, the pattern and severity of the seizure changed rapidly with increasing age in 3 stages: 1) clonic movements of forelimbs, 2) clonus of all limbs, 3) clonus preceded by tonic flexion. Between 20-30 days, the tonic extensor component of a maximal seizure was most prominent. Alterations in the pattern and severity of the seizure response with age were related to the stages of development of brain carbonic anhydrase: at 1-10 days, low levels of enzyme activity; 10-20 days, a rapid 4-fold increase in activity; 20-30 days, maximal activity. Only minimal clonic seizures could be induced in animals with low levels of brain carbonic anhydrase; maximal tonic seizures were

observed after high levels of enzyme activity developed. These observations are in accord with the demonstration that acetazolamide prevents the tonic electroshock seizure but does not raise the threshold to minimal clonic seizures; its action is related directly to the inhibition of brain carbonic anhydrase (Fed. Proc. 1954, 1955). Other factors of possible significance in the development of seizure patterns in the newborn will be discussed.

1382. EFFECTS OF TRANQUILIZING DRUGS ON CEREBRAL CORTICAL REACTIONS TO EPINEPHRINE. Bruno Minz. Research Dept., Osawatomie State Hosp., Osawatomie, Kan.

Previous investigations have shown that a short local application (15-30 sec) of a concentrated solution of epinephrine (5%) on the frontoparietal area of the cerebral cortex of the rabbit elicits an immediate rise in systemic blood pressure (Minz, Buser, Albe-Fessard. J. Pharmacol. & Exper. Therap. 110:38, 1954). It has been established through a detailed pharmacodynamic analysis that this effect is not due to a diffusion of epinephrine from the cortex into the general circulation, but to an indirect action on hypothalamic structures which may react by liberating a specific pressor agent. It thus seemed of interest to investigate possible modifications of cortically induced hypertension by tranquilizing drugs. Reserpine and Chlorpromazine were selected and used in these experiments. After an i.v. injection of Reserpine, the blood pressure response to cortically applied epinephrine was amplified, whereas Chlorpromazine was found to have an opposite effect. The latter decreases the reaction of the cortex in normal rabbits as well as in animals which have been pretreated with Reserpine. Under the action of Reserpine the cortex, which is normally insensitive to the application of a 1% solution of epinephrine, reacts with pressor impulses after a local administration of a 0.5 and even a 0.1% solution. This sensitization becomes evident about 4 hr after treatment and persists for 3 days; it suddenly drops down to normal levels the 4th day. Possible correlations between the observed effects and the role of serotonin and other centrally acting agents are discussed.

1383. PHARMACOLOGICAL PROPERTIES OF SOME NEW SUBSTITUTED PYRIDINE AND PIPERIDINE UNSYMMETRICAL BIS-QUATERNARY HYPOTENSIVE AGENTS. Joseph H. Mirsky,* Martha D. Napoli* and Thomas B. O'Dell. Irwin, Neisler Research Labs., Decatur, Ill.

Pharmacological studies are described with a group of new substituted pyridine and piperidine unsymmetric bis-quaternary compounds. In the dog, i.v. administration of such compounds produced a hypotensive effect which was characterized by having a moderately slow onset, with the blood pressure fall occurring over a 30-45 min period. The effect of relatively minor structural variations on the hypotensive potency was quite marked and will be discussed. Although the sympathetic ganglionic blocking activity of these compounds is of the order of that of hexamethonium, their effectiveness in lowering blood pressure is evident at dramatically smaller doses as low as 10-25 γ/kg. This group of compounds particularly well demonstrates that the hypotensive action is not directly correlated with the peripheral autonomic effects of these compounds.

1384. INFLUENCE OF CERTAIN NON NARCOTIC ANALGESICS ON THE OXYGEN UPTAKE OF RAT LIVER SLICES. Tom S. Miya and Bob West.* Purdue Univ., Lafayette, Ind.

Bilateral hind leg tourniquets wound tightly and tied as high up on the legs as possible caused a marked increase in oxygen consumption of exicsed liver slices from animals subjected to this treatment. Experiments with acetylsalicylic acid, 600 mg/kg and salicylamide, 200-300 mg/kg indicate that these drugs do not block the increased oxygen uptake due to tourniquet shock. Orally administered acetylsalicylic acid, 600 mg/kg and 700 mg/kg resulted in increases in oxygen uptake of the excised liver slices of 17.5 and 27.1% respectively. Sodium salicylate, 400 mg/kg produced an increase of 26%. The minimal effective dose for increased oxygen consumption by salicylamide was 300 mg/kg. The oral administration of acetophenetidin, 750 mg/kg resulted in an increase of 17.9%. At 200 mg/kg the pyrazolon derivatives, aminopyrine and antipyrine, produced increases of 19.6 and 17.9% respectively in oxygen uptake. Slight increases were also noted after the oral administration of morphine sulfate, 10 mg/kg and sodium phenobarbital, 25 mg/kg. The in vitro addition of sodium salicylate, salicylamide, acetylsalisylic acid, and antipyrine to the liver slices in the Warburg flasks in molar concentrations from 0.00005 to 0.001 M did not increase oxygen consumption. Morphine sulfate in concentrations of 1×10^{-3} M did not exert an effect on oxygen uptake. Similar results were obtained with sodium phenobarbital at concentrations ranging from 1×10^{-6} to 1×10^{-3} M. The observed increase of oxygen consumption following pretreatment with these drugs does not appear to be due to an activation of the adrenal pituitary axis since pretreatment with ACTH and cortisone did not alter oxygen uptake.

1385. EFFECT OF IONIZATION ON CARDIAC STIMULATING PROPERTIES OF THEOPHYLLINE. Joanne I. Moore and Harold F. Hardman (intr. by Maynard B. Chenoweth). Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

The hypothesis is presented that the concentration of unionized theophylline presented to the perfused turtle heart is the critical factor in determining the positive inotropic activity of this drug. The ratio between the ionized and unionized forms of theophylline was varied over a wide range by altering the pH of the perfusion media to the turtle heart. It was observed that the ionized form of theophylline did not stimulate the turtle heart even with doses 10 times greater than the concentration of unionized theophylline required to produce a 30% stimulation. The ratio of ionized to unionized forms of the drug present in our system is dependent on the pH of the perfusion media and the pK_a of the drug. Theophylline has a pK_a of 8.70, so that at the biological pH of 7.40, 95% of a given dose would exist in the unionized form, while the remaining 5% would be ionized. By contrast, 8-chlorotheophylline, 8-bromotheophylline, and 1-3 dimethyluric acid exist primarily in the ionized form at pH 7.40 because of their lower pK_a values. The difference in the pK_a value of the #8 substituted derivatives and theophylline per se is offered as a partial explanation for the almost complete lack of positive inotropic activity observed with the #8 substituted derivatives in several species of animals.

1386. EFFECT OF CHLORPROMAZINE ON OXYGEN CONSUMPTION BY TISSUE SLICES. Albert S. Moraczewski* and Kenneth P. DuBois. Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

It has been reported that chlorpromazine decreases the oxygen consumption by rat brain slices

at concentrations between 5.6×10^{-5} M and 7×10^{-4} M but does not affect the respiration of rat liver slices (Minerva anestesiol. 19:278, 330, 1953). Norman and Hiestand have recently found (Proc. Soc. Exper. Biol. & Med. 90:89, 1955) that chlorpromazine produces a marked hyperglycemia in hamsters and mice but has no effect on the blood glucose of rats. This finding stimulated our interest in ascertaining whether the intermediary metabolism of liver slices from various species differs in susceptibility toward inhibition by chlorpormazine. We, therefore, undertook a comparison of the effects of this drug on oxygen consumption by tissue slices from rats, mice and guinea pigs. These experiments demonstrated that the respiration of liver slices from rats and guinea pigs is not affected by 1×10^{-3} M chlorpromazine; however, the endogenous Q_2O of mouse liver was 48% inhibited by this concentration of chlorpromazine. The normal stimulating effect of oxalacetate, succinate and pyruvate on oxygen consumption by mouse liver slices was reduced 22%, 34% and 60% respectively by 1×10^{-3} M chlorpromazine. The sulfoxide of chlorpromazine exerted no significant inhibitory effect on the respiration of mouse liver slices. The endogenous respiration of brain slices from mice, rats and guinea pigs was inhibited 65%, 58 58% and 36% respectively by 1×10^{-3} M chlorpromazine

1387. HISTAMINE-LIKE ACTIONS OF OXYPANAMINE, AN ALKALOID DERIVED FROM ORMOSIA PANAMENSIS. Neil C. Moran, Gertrude P. Quinn* and William M. Butler, Jr.* Lab. of Chem. Pharmacology, Natl. Heart Inst. and Dept. of Pharmacology, Emory Univ., Ga.

Oxypanamine (NHI-196), an N-oxied of panamine derived from Ormosia panamensis, produced, in the dog, histamine-like actions characterized by a 30-120 sec delay in onset after i.v. injection, long duration and tachyphylaxis. There is no release of histamine into the blood (Moran et al. Fed. Proc. 15:462, 1956). The present experiments were designed to help clarify these histamine-like effects. The hypotensive action in anesthetized dogs was greatly reduced by large doses of carbinoxamine but not by tripelennamine or pyrilamine, while the rise in pulmonary artery pressure and intratracheal pressure (bronchoconstriction) was completely abolished by all 3 antihistamine agents. Oxypanamine (0.5 mg/kg produced transient moderate increases in intestinal motility in anesthetized, atropinized, vagotomized dogs; but there was no latent period of tachyphylaxis, nor was the gut stimulation inhibited by histamine-blocking doses of carbinoxamine. In an unanesthetized dog with a Heidenhain pouch there was no significant secretion of gastric acid with doses of oxypanamine (0.5 mg/kg) which produced marked cutaneous erythema and other evidence of pharmacological action, whereas histamine produced marked acid secretion before and after oxypanamine. Maximal contractions of the isolated ileum of the guinea pig were obtained with oxypanamine at 500 μg/ml concentration compared with 0.25 μg/ml of histamine dihydrochloride. Histamine-blocking doses of tripelennamine produced variable degrees of inhibition of oxypanamine. Tachyphylaxis was observed occasionally with repeated doses of oxypanamine on the isolated gut inspite of washing between doses. In these cases the response to histamine was reduced. It is concluded that the bronchial and vascular, but not the gastrointestinal, actions of oxypanamine are histamine-like (i.e., blocked by antihistamine drugs), but that the action is not direct on histamine receptors (latent period and lack of direct vasodilating action, Moran et al. Ibid.) and does not involve release of histamine into the blood.

1388. INFLUENCE OF STEROID HORMONES ON OXIDATION OF ORGANIC THIOPHOSPHATES. Sheldon D. Murphy* and Kenneth P. DuBois. Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

Recent studies in this laboratory (J. Pharmacol. & Exper. Therap. In press) demonstrated that the dimethyoxy ester of benzotriazine dithiophosphoric acid (DBD; Guthion) is oxidized to a cholinergic oxygen analogue by a pyridine nucleotide-linked microsome enzyme in liver. Development and application of a quantitative method for measuring the activity of this enzyme system demonstrated that the enzyme activity of the livers of adult male rats is 2 to 3 times greater than that of adult female rats. However, female rats are more susceptible to the acute toxic effects of DBD suggesting that the conversion of thiophosphates to anticholinesterase agents is under hormonal control in vivo. Experiments were, therefore, undertaken to ascertain the effect of various steroid hormones on the oxidation of thiophosphates. The enzyme activity of male rat liver was 50% inhibited by 8.1×10^{-5} M testosterone, 7.5×10^{-6} M progesterone, 9×10^{-6} M diethylstilbexterol and 1×10^{-4} M desoxycorticosterone in vitro. Estradiol, estrone and cortisone produced no significant inhibition of the enzyme activity. The livers of young male and female rats (30 days old) exhibited equal low enzyme activity within the range for adult females. After partial hepatectomy the enzyme activity of the regenerating liver of male rats was only 22% of normal in 4 days. At 24 hr after the i.p. injection of 3-methylcholanthrene (25 mg/kg the enzyme activity of regenerating liver of adult males and the livers of adult female rats was markedly increased. Administration of ethionine (100 mg/kg) 1 hr before methylcholanthrene prevented the effect by the latter compound.

1389. QUANTIFICATION OF MODERATE DEGREES OF DRUG-INDUCED ANALGESIA, BY A MODIFICATION OF THE WOOLFE-MACDONALD TECHNIQUE. Joe B. Nash, Robert G. Brown, Delores A. Jamison and Marjorie E. Nielsen (intr. by G. A. Emerson). Dept. of Pharmacology and Toxicology, Univ. of Texas Med. Branch, Galveston.

Tests of changes in reactiontime of Swiss-W mice after treatment with Shigella neurotoxin (Brown. Thesis, University of Texas, 1957) indicated that small degrees of analgesia might be quantified by statistical treatment of results of quantitative measurements made by a modification of the Woolfe-MacDonald method (J. Pharmacol. & Exper. Therap. 80:300, 1944). This indication is confirmed by studies of representative analgetics. Dose-effect relations are presented for morphine sulfate, 5-30 mg/kg, codeine phosphate, 20-60 mg/kg, meperidine hydrochloride, 10-60 mg/kg, dihydromorphinone hydrochloride, 1-3 mg/kg, and dihydrocodeine bitartrate, 10-50 mg/kg. The results demonstrate that small differences in analgetic effect may be differentiated as well, or better, by this exceedingly simple and inexpensive technique as by those far more elaborate.

1390. BIOSYNTHESIS OF CHOLESTEROL IN THE CENTRAL NERVOUS SYSTEM. Harold J. Nicholas (intr. by Lawrence Peters). Dept. of Obstetrics and Gynecology, Univ. of Kansas Med. Ctr., Kansas City.

According to the literature, nervous tissue of

young animals readily incorporates acetate into the cholesterol molecule, but mature nervous tissue lacks this capacity. To explore this area 2-C^{14}-sodium acetate was injected subcutaneously into 4-day old and 30-day old Sprague-Dawley rats and incubated with homogenized and minced brain tissue. The incubating mixture contained Mg ions, DPN, ATP and nicotinamide in a phosphate buffer at pH 6.5. In the 4-day old rats incorporation of C^{14} into brain cholesterol was appreciable by injection or incubation with minced tissue. In the 3-day old rats slight but definite incorporation occurred in the minced brain as measured by C^{14} activity in the isolated cholesterol dibromide. By direct intracerebral injection of 2-C^{14}-acetate into 30-day old rats under light ether anesthesia, cholesterol dibromide was obtained as radioactive as that from incubation of minced 4-day old brain tissue. While the mechanism of this conversion is not clear, the presence of some "barrier" in mature brains inhibiting the incorporation of acetate into cholesterol seems indicated. All preparations synthesized nonsaponifiable material not precipitated by digitonin. In the 30-day old brains much of this material could be eluted from alumina with petroleum ether and was therefore nonpolar in nature.. All C^{14} determinations were performed with a windowless counter. (Supported in part by Grant No. 133 from the Natl. Multiple Sclerosis Soc.)

1391. FACTORS DISTINGUISHING CLASSICAL COMPETITIVE FROM NONEQUILIBRIUM DRUG ANTAGONISM. Mark Nickerson and Elsie H. Morthan.* Dept. of Pharmacology and Therapeutics, Univ. of Manitoba Faculty of Medicine, Winnipeg, Canada.

Nonequilibrium antagonism differs from classical competitive antagonism in that although agonist and antagonist combine with the same tissue receptors in both, nonequilibrium antagonists react chemically with the receptors, and therefore, are not in mass-action equilibrium with the agaonist. Consequently, dose-effect data for nonequilibrium antagonists would be expected to appear noncompetitive when plotted by the classical methods of Gaddum and of Linweaver and Burk. Plots of this type may reliably differentiate classical competitive from noncompetitive or from nonequilibrium inhibition in relatively simple enzyme systems, but are not applicable to drug antagonisms in surviving tissue because here the agonist-receptor combination does not appear to be the limiting factor in the response, i.e., a maximal response can occur when only a small percentage of the vailable receptors are occupied by agonist (Nature 178:697, 1956). A more reliable test to distinguish between nonequilibrium and classical competitive antagonism can be based on the fact that two distinct factors are involved in determining the time course of a blockade. The onset and duration of action of classical competitive antagonist is largely dependent on penetration into and escape from the biophase (immediate environment of the receptors), whereas the duration of action of nonequilibrium antagonists is largely determined by the stability of the antagonist-receptor complex. Consequently, a series of intermittent exposures to a classical competitive antagonist should produce less blockade than a single exposure of equal total duration, whereas intermittent exposure to a nonequilibrium antagonist should produce more blockade. This expectation has been confirmed by in vitro studies with both antihistaminics and adrenergic blocking agents.

1392. METABOLISM OF 4-C^{14}-LABELED DIPHENYLHYDANTOIN IN RATS. E. L. Noach,* Dixon M. Woodbury and L. S. Goodman. Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

C^{14}-labeled diphenylhydantoin (Dilantin) with a specific activity of about 0.35 μC/mg was injected into rats at a dose of 22 mg/rat. The animals were killed after 3 and 30 min, 1, 1-1/2, 2, 4, 8, 16 and 24 hr. Dilantin and/or its products were determined by both chemical and radioactive procedures in samples of plasma, brain, salivary gland, liver, kidney, perirenal fat, muscle, urine and gastrointestinal tract from cardia to rectum. Dilantin was rapidly concentrated in all organs investigated. For the first hour after administration, concentrations declined, but they showed a second peak after 1-1/2 hr. After 24 hr, little of the compound was still detectable chemically and only traces of radioactivity remained. No storage of Dilantin in specific tissues could be detected. During the first hour after administration, the highest concentrations were found in liver and salivary glands. Although little metabolic conversion occurred in salivary glands, considerable conversion to a chemically different compound was noted in liver. This began within minutes after administration, and preliminary evidence indicates subsequent biliary excretion. By 24 hr, only 2% of the injected Dilantin had been excreted unchanged in the urine. However, about 20% of the radioactivity had been excreted. The metabolic pathways involved will be discussed. (Supported in part by Grant B-381, Natl. Inst. of Health, PHS, and by contract number 19(600)921, USAF.)

1393. INFLUENCE OF SEVERAL THIOL COMPOUNDS ON THE MORTALITY AND SURVIVAL TIME OF X-IRRADIATED MICE. J. F. Noble,* V. J. Plzak,* R. M. Dowben* and J. Doull. U.S. Air Force Radiation Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

A large number of thiol-containing compounds were tested for prophylactic and therapeutic effectiveness against radiation lethality in adult male Carworth Farms mice exposed to 800 r of whole body X-irradiation (250 kvp, 15 ma, target-skin distance 75 cm, dose rate 37 to 40 r/min). Preliminary toxicity studies were carried out with each compound to determine maximum dosage levels which could be given acutely or chronically to mice without causing mortality. The compounds were dissolved in water or propylene glycol and given i.p. In the acute studies the compounds were administered immediately before the X-ray exposure and in the chronic studies the mice were treated for 1 wk before and 1 wk after irradiation. Control animals were irridated with each group of treated mice. Observations on the mortality and weight loss were made daily for 1 wk prior to and for 30 days following the X-ray exposure. Prolongation of survival time and decreased mortality occurred following the acute administration of ethyl isothiourea, 2-benzoxazolethiol, 2-amino-6-methylbenzothiazole and diethyl dithiocarbamic acid to irradiated mice. Significant though lesser protective effects were obtained with 2-benzthiazolethiol, dibenzothiophene, 1,3-di-n-butyl-2-thiourea, alpha-mercaptoacetanilide and 10-(alpha-pyrrolidinopropionyl)-phenothiazine. None of the compounds protected mice against radiation lethality when given chronically and the protective activity in all cases was less than that obtained following preirradiation administration of beta-mercaptoethylamine, beta-aminoethylisothiouronium or para-aminopropiophenone.

1394. EFFECT OF SECRETIN ON DIASTASE IN SERUM AND URINE IN NORMAL,

PANCREATECTOMIZED AND PANCREATIC FISTULA DOGS. Martin M. Nothman and Allan D. Callow.* Med. and Surgical Depts. of the New England Med. Ctr. and Tufts Univ. School of Medicine, Boston, Mass.

Secretin given to normal dogs does not increase the values for diastase in serum and urine. When the pancreas in these dogs is removed the concentration of diastase in serum and urine does not change. Secretin injected after the operation has no effect on the diastase values. Ligation of the pancreatic ducts results in an immediate increase of the diastase in blood followed by a rise of the diastase in the urine. The values for serum and urinary diastase rise further when Secretin in injected. When the pancreas is removed a few days after duct ligation, they fall to their original values cut do not disappear. Establishment of a pancreatic fistula after tying of the ducts has the same effect as the removal of the pancreas after duct ligation: that is, the values for urinary and serum diastase return to their original level. Secretin given after the pancreatic fistula has been performed, does not influence the values for diastase in urine or serum but does increase the amount of diastase secreted through the fistula 5-8 times. Considerable amounts of diastase are present in the bile of dogs. After ligation of the pancreatic ducts or pancreatectomy the enzyme is still found in the duodenal juice of the animals. From the presented facts we conclude: The diastase present regularly in serum and urine is not of pancreatic origin. Since considerable amounts of diastase have been found in bile the liver has to be considered as a possible source. The increased values of diastase which can be detected in serum and urine under pathological conditions are derived from the pancreas. Studies on diastase in serum and urine after removal of the liver are in progress. (Supported by a grant-in-aid from the Damon Runyon Fund for Cancer Research.)

1395. PHARMACODYNAMIC STUDIES ON MECHANISM OF HYPOTENSIVE ACTION OF SUBTUTED PYRIDINE UNSYMMETRICAL BISQUATERNARY COMPOUNDS. Thomas B. O'Dell and Martha D. Napoli.* Irwin, Neisler Research Labs., Decatur, Ill.

The compound used to carry out these studies was 1-[4-(1-methyl-3-indoleethyl)-pyridinium]-3-(trimethylammonium)-propane dichloride (IN 391). The sustained hypotensive effect of this compound appears to involve some central site. This conclusion is based on both direct and indirect evidence obtained using dogs and cats. The procedures employed and the results obtained will be presented. These mechanism studies with IN 391 show it to be a very potent hypotensive agent with a marked degree of selectivity of action. Evidence will also be presented to show that the effects of this compound, when administered orally, can be potentiated by increasing absorption from the G.I. tract through the use of an additive agent.

1396. COLLAGEN CONCENTRATION OF HUMAN MYOCARDIUM. Donald E. Oken and Robert J. Boucek (intr. by Ralph Jones, Jr.). Dept. of Medicine, Univ. of Miami School of Medicine and Howard Hughes Med. Inst., Miami, Fla.

The collagen concentration of the 4 cardiac chambers was determined on a random series of 83 normal and diseased hearts obtained at necropsy from individuals of different ages. Endocardium and epicardium and eipcardium were stripped from some ventricles and all atria prior to analysis. The collagen values were calculated from the chemical determination of hydroxyproline and expressed as grams percentage of total protein. The mean collagen concentration of the right ventricle (7.5±1.9) was found to be significantly higher than that of the left ventricle (5.5 ±0.82). The right atrium similarly had a significantly higher value (16.5±4.4) than the left atrium (13.5±3.2). The mean concentrations of both atria were more than double the values for the ventricles. Collagen concentration was statistically higher in the first decade of life than in subsequent decades which had relatively constant collagen values. Since the collagen concentration of the left ventricle in hypertrophy was not different from the normal ventricle, it appears that there was an increase in collagen to the same degree as the increase in muscle mass. Hearts containing areas of infarction had an increased collagen concentration only in tissue obtained from or near the infarcted areas. Coronary atherosclerosis alone was not found to result in an increased collagen concentration in either ventricle. No difference was found between the collagen concentrations of the myocardium of males and females.

1397. ACETYLATION OF HYDRAZINE AND ITS RELATION TO HYDRAZINE LETHALITY. John F. O'Leary, Aili H. Oikemus and Lawrence J. Edberg (intr. by J. H. Wills). Chem. Warfare Labs., Army Chem. Ctr., Md.

It has been observed that certainly-lethal doses of hydrazine given to mice i.v. require at least 7 min to kill the animals, which die in convulsions. It is not known whether this latent period is due to time required for hydrazine to diffuse to its site of CNS action, or to time required for chemical alteration of hydrazine to an active form. As previously reported, barbiturates, particularly phenobarbital, are protective to some degree against such hydrazine deaths. Marks has reported that barbiturates are able to inhibit enzymatic acetylation of amines by pigeon liver in vitro, and that phenobarbital is one of the more potent longacting barbiturates in this respect. The possibility therefore existed that the protective mechanism of phenobarbital against hydrazine was the inhibition of the acetylation of hydrazine. This hypothesis required the assumption that acetylated hydrazine is the ultimate "active" form of injected hydrazine, and hence should be more toxic than hydrazine itself. This assumption was tested by administering mono-acetylated or di-acetylated hydrazine to mice i.v. in doses which were equi-molar with respect to lethal hydrazine doses. Neither acetylated compound was more toxic than hydrazine with regard to dose or time to death, and hence acetylation inhibition does not appear to be the mechanism of barbiturate protection against hydrazine CNS effects.

1398. EFFECT OF RESERPINE UPON THE MAMMALIAN HEART. M. K. Paasonen* and O. Krayer. Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

Reserpine increases the heart rate in the heart-lung preparation of the dog (Plummer et al. Ann. New York Acad. Sci. 59:8, 1954; Krayer and Fuentes, Fed. Proc. 15:709, 1956). Initially, the heart rate increase resembles that caused by a continuous infusion of epinephrine or norepinephrine (NE). In an attempt to elucidate the mechanism, the changes of the NE content of the rat heart in situ under the influence of reserpine were investigated. NE was determined biologically, using the responses of the cat blood pressure and of the isolated rectal caecum of the chicken, after extraction by von Euler's metho

(Acta physiol. scandinav. 16:63, 1948). Reserpine (5 mg/kg i.p.) releases NE from the rat heart. After 2 hr, only 1/10, or less, of the control amounts are present. After 72 hr, the NE content is still far below the control values. Since depletion of NE was shown to occur in the cat hypothalamus (Holzbauer and Vogt. J. Neurophysiol. 1:8, 1956), this reserpine effect may be a more general phenomenon.

1399. SERUM GLUTAMIC-OXALACETIC TRANSAMINASE ACTIVITY IN IRRADIATED ANIMALS. D. F. Petersen, C. C. Lushbaugh and P. Lee.* Los Alamos Scientific Lab. Univ. of California, Los Alamos, N. M.

The recent demonstration of altered serum glutamic-oxalacetic transaminase (SGO-T) activity following various types of tissue injury suggested that SGO-T measurements might be applicable to the investigation of early tissue injury following exposure to ionizing radiations. This hypothesis was tested employing rabbits subjected to total body and localized irradiation of the liver, heart, kidney and gut. Immediatly following administration of 644 r of total body irradiation, SGO-T activity was 159% of normal and gradually increased to 223% of normal during the next 5 hr. Immediately after exposure of the liver, gut and kidney to 554 r, SGO-T values increased to approximately twice the preirradiation level and increased progressively during the following 6 hr to 276%, 328% and 362%, respectively. A smaller increase to 226% was noted after administration of a similar dose to the heart. Rapid reversibility of these changes was evidenced by a return to essentially normal values within 24 hr after irradiation. It was further noted that dose rates of less than 10 r/min failed to produce the change. The extremely rapid rise of transaminase activity in the peripheral blood may indicate that the initial biochemical injury to the cells of irradiated animals occurs within a matter of minutes after exposure.

1400. ACETYLCHOLINE CONGENORS: STRUCTURE ACTIVITY OF NICOTINIC AND MUSCARINIC ACTION. Carl C. Pfeiffer and Edmund W. J. De Maar.* Div. of Basic Health Sciences, Emory Univ., Ga.

The studies of Wilson and Bergman on cholinesterase have established the geometrical concept of separate cationic and esteratic sites on this enzyme. A similar dual concept may be postulated for the acetylcholine "receptors" to explain nicotinic and muscarinic action wherein the former are mainly influenced by the cationic head and the latter by the ester end of acetylcholine. While both ends of the molecule are needed for maximal activity some degree of action can be obtained with the free ends, i.e., ganglionic stimulation with Tetramethyl ammonium and muscarinic effects with brompyruvic acid. Since some nicotinic effects are opposed to muscarinic effects balance may be found between these two effects in some congenors. Compounds were arranged, regardless of molecular structure, in an order of increasing muscarinic potency. Compounds arranged in this order show a decrease in nicotinic potency. A high degree of muscarinic potency was found to be more associated with definite structural arrangements for the esteratic end of the molecule than for the cationic end of the molecule. Nicotinic potency is found to be more associated with basic requirements for the cationic end. It appears from these findings that the receptors for muscarinic and nicotinic effects do differentiate between the esteratic end and the cationic end of the molecule, instead of being dependent only on strict overall structural configurations of the molecule.

1401. CARDIOVASCULAR AND RESPIRATORY EFFECTS OF FLUOTHANE ANESTHESIA IN DOGS. C. B. Pittinger, J. P. Long, D. C. Watland* and S. C. Cullen. Dept. of Surgery, Div. of Anesthesiology, and Dept. of Pharmacology, State Univ. of Iowa College of Medicine, Iowa City.

Fluothane, 2-bromo-2-chloro-1,1,1-trifluoroethane, is a new volatile anesthetic agent which is 100% potent and non explosive. The abstract of laboratory findings by Raventos (Abstr. XX Internat. Physiol. Congress, 1956. p. 754) and two clinical reports by Johnstone (Brit. J. Anesth. 28:392, 1956) and Bryce-Smith and O'Brien (Brit. M. J. 2:969, 1956) suggested the need for investigating cardiovascular and respiratory effects of the drug since hypotension, bradycardia and respiratory alterations has been observed during Fluothane anesthesia. Nonpremedicated dogs were prepared during light Fluothane anesthesia for determinations of heart rate, arterial blood pressure, pulse pressure, respiratory rate, tidal and minute volumes and recording of electrocardiograms. The animals were intubated orotracheally and connected to a nonrebreathing system. After awakening, observations were made and then repeated during varying depths of anesthesia. With increasing depth of anesthesia, blood pressure, pulse pressure and heart rate decreased, but these effects were partially counteracted by the intravenous administration of atropine. No ominous changes were observed in the electrocardiographic tracings. Respiratory rate and minute folume declined with increasing depth of anesthesia after an initial stimulatory phase associated with excitement. Additional studies of cardiovascular and respiratory effects of Fluothane will be reported.

1402. COMPARATIVE HEPATOTOXICITY OF SEVEN HALOGENATED HYDROCARBONS. G. L. Plaa,* E. A. Evans* and C. H. Hine. Dept. of Pharmacology and Exptl. Therapeutics, Univ. of California School of Medicine, San Francisco.

The hepatotoxic potency of a series of aliphatic halogenated hydrocarbons of low molecular weight was evaluated by measurement of prolongation of pentobarbital anesthesia. Pentobarbital was given to mice in a single i.p. dose of 45 mg/kg, 24 hr after a single hydrocarbon injection. In 270 control (pentobarbital only) observations, the mean sleeping time was 1.5±4.1 minutes; only 22% of the controls actually lost their righting reflex. In the experimental animals, a sleeping time greater than 10 min was considered significant. It was possible to produce a significant prolongation of sleeping time with all the compounds at a sublethal dose. The hepatotoxic ED in moles per kilogram was 5.5×10^{-4} for carbon tetrachloride, 1.8×10^{-3} for chloroform, 1.1×10^{-2} for trichloroethylene, 2.8×10^{-2} for tetrachloroethylene. The toxicity of 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethane increased in that order, and doses which produced significant increase in sleeping time in half the animals also produced death. At necropsy, livers of animals given lower doses were grossly and microscopically normal; at higher doses there was increasing discoloration and friability. Microscopically varying degrees of central necrosis were seen, correlating well with the observed prolongation of sleeping time. Most animals receiving chloroform and trichloroethane showed renal tubular necrosis, even at doses which

did not significantly prolong the sleeping time; these changes were not apparent grossly. (Supported in part by a grant from E. I. DuPont de Nemours Co.)

1403. PROLONGED ANTIHISTAMINIC ACTION OF A SERIES OF THIANAPHTHINDOLES. A. J. Plummer, W. E. Barrett,* R. Rutledge* and L. Werner.* Research Dept., Ciba Pharmaceutical Products Inc., Summit, N. J.

Antihistaminic activity of gradual onset and marked duration was associated with a number of N-substituted dialkylaminoalkyl thianaphthindole derivatives. The activity of the series may be further improved by appropriate substitution on the ring structure with chlorine or alkyl groups. The antagonism to histamine was demonstrable with both the isolated guinea pig ileum and with the intact guinea pig after the intravenous administration of histamine. In the latter preparation excellent protection against a lethal dose of histamine was afforded for a period of 9-12 hr by the oral administration of 5.8 mg/kg of 8-Methyl-10-(2-dimethylaminopropyl) thianaphthindole, one of the more active compounds. Protection was also demonstrated against anaphylactic shock and death in the guinea pig sensitized to horse serum. Specificity of effect was indicated by the lack of significant effects on blood pressure, respiration, or the central nervous system in effective antihistaminic doses. Anticholinergic action was not noted. Sedative effects were not observed in mice, cats or dogs. Acute toxicity was moderate, with an i.v. LD_{50} in the white rat of 60 mg/kg. No specific toxic effects nor unfavorable action on the blood picture has been noted with chronic administration of the more active derivatives.

1404. TIME COURSE STUDIES OF EFFECT OF ESTROGENS ON PITUITARY GONADOTROPHIC FUNCTION. Gordon O. Potts and Arthur L. Beyler (intr. by E. W. Dennis). Sterling-Winthrop Research Inst., Rensselaer, N. Y.

17α-Ethinyl-5-androstene-3β,17β-diol and its esters are of interest because of their estrogenic and pituitary inhibitory activities. Representative members of this series have been compared with estradiol-17β, diethylstilbesterol and esters thereof with respect to their ability to inhibit the pituitary. The onset, degree and duration of pituitary inhibition, measured in terms of sex accessory organ atrophy and pituitary gonadotrophin content were assessed on a weekly basis up to 6 wk. All compounds within a series were administered as single s.c. injections to male Sprague-Dawley rats (170-190 gm) at dose levels which were equimolar in terms of the free alcohol. The maximum pituitary inhibition together with week of occurrence obtained for each test estrogen were: ethinylandrostenediol, 53% at 2 wk; ethinylandrostenediol 3-propionate, 75% at 2 wk; ethinylandrostenediol 3-cyclohexylpropionate, 76% at 3 wk; estradiol-17β, 23% at 1 wk; estradiol-monobenzoate, 52% at 1 wk: estradiol dipropionate, 60% at 2 wk; estradiol cyclopentylpropionate, 80% at 4 wk; diethylstilbesterol, 22% at 5 wk; diethylstilbesterol dipropionate, 77% at 4 wk; diethylstilbseterol dipalmitate, 81% at 4 wk. Ethinylandrostenediol 3-cyclohexylpropionate, diethylstilbesterol dipropionate, diethylstilbesterol dipalmitate and estradiol cyclopentylpropionate exhibited sustained activity over the 6-wk period. An observed lack of parallelism between pituitary gonadotrophic content and time of change in sex accessory organs indicated that strucutre of the test estrogen and rate of synthesis and release of the trophic hormone are correlative.

1405. INFLUENCE OF PENTOBARBITAL ON VENTRAL ROOT REFLEX DISCHARGES AND ON INTRACELLULAR POTENTIALS RECORDED FROM SINGLE MOTONEURONS. James B. Preston. Dept. of Physiology, State Univ. of New York, College of Medicine, Syracuse.

Reflex discharges initiated by stimulation of a hindlimb nerve were recorded from the appropriate cut ventral root in acute spinal cat preparations. The average 50% depressant dose of pentobarbital given i.v. was 4.4 mg/kg for the polysynaptic discharge, 11.0 mg/kg for the monosynaptic reflex discharge, and 17.0 mg/kg for the potentiated monosynaptic discharge. Pentobarbital in doses of 2.5-4.0 mg/kg produced a slight increase in the height of the monosynaptic discharge in approximately 50% of the preparations. Preliminary data in another series of experiments further elaborates the influence of pentobarbital on the motoneurons of the spinal cord. Intracellular recordings form motoneurons were obtained by means of KCl filled glass micropipettes. Pentobarbital produced a failure of motoneuron spike discharge and depression of the snyaptic potential. In some units antidromic activation of the motoneuron was blocked at the same dose level which blocked orthodromic activation. These data suggest that pentobarbital has a postsynaptic site of action.

1406. DIGOXIN EFFECT ON HEART ATPASE. Charles D. Proctor and Gioia S. Mueller (intr. by Y. T. Oester). Dept. of Pharmacology and Exptl. Therapeutics, Stritch School of Medicine of Loyola Univ., Chicago, Ill.

Prior work carried out in this laboratory has indicated an in vitro inhibitory effect of digitoxin on rabbit heart calcium activated adenosinetriphosphatase (ATPase). Such inhibition was demonstrated consistently only when adenosinetriphosphate (ATP) levels somewhat lower (2 mM final concentration) than those usually used (3 mM final concentration) in ATPase reaction mixtures were employed (Proctor et al. Ann. New York Acad. Sci. 62:377, 1955). Using methods similar to those employed in the previous study of digitoxin inhibition of ATPase the effect of digoxin on ATPase from rabbit heart has been investigated. Digoxin, present at 4 μg/ml concentration, was incubated with 30% W/V heart homogenates at 37°C for 1/2 hr prior to dilution of the homogenate to 1% W/V. Diluted homogenates were immediately added to ATPase reaction mixtures and the enzyme reaction allowed to proceed for 15 min at 37°C. Duplicate determinations were made for controls (homogenates incubated without digoxin) and for the digoxin treated homogenates. Results obtained indicate that digoxin effects in vitro inhibition of the ATPase in a range of 8-16%. (Supported by a grant from the Chicago Heart Assoc.)

1407. COMPARATIVE PHARMACOLOGICAL STUDIES OF CROTALUS ADAMANTEUS AND NAJA FLAVA VENOMS. Jack L. Padomski and William B. Deichmann. Dept. of Pharmacology, Univ. of Miami School of Medicine, Coral Gables, Fla.

Although there is much information in the literature on the pharmacological and enzymatic actions of snake venoms, a great deal of it is contradictory. It was felt desirable to do a careful comparative pharmacodynamic study on two venoms of widely divergent actions, Naja flava (cobra) and C. adamanteus (rattlesnake). C. adamanteus produces in the anesthetized dog and rabbit a profound and sustained fall in blood pressure. Naja flava, even in toxic doses, does not affect the blood pressure. Since both venoms have

high lecithinase A potency, it appears that this enzyme activity of snake venom is not responsible for the hypotensive action. Moreover, with neither venom was it possible to demonstrate hemolysis in the blood of even severely poisoned animals. Neither of the venoms have any adverse effect on the dog or rabbit electrocardiogram. The sequence of events preceding death with C. adamanteus in the anesthetized dog are: the blood pressure falls toward zero: the respiration stops· cardiac arrest occurs. With Naja flava, respiration and blood pressure fall simultaneously; then, cardiac arrest occurs. Further studies on the blood of poisoned animals were conducted. C. adamanteus produced marked hemorrhages. The blood clotting time and prothrombin time were markedly increased. This venom also produced a dramatic leukocytosis. The results of electroencephalographic studies on dogs will also be presented.

1408. DRUG TRANSFER FROM BLOOD TO CEREBROSPINAL FLUID. David P. Rall and Charles G. Zubrod. Natl. Cancer Inst., Bethesda, Md.

There has long existed a need for a quantitative approach to the study of transfer of drugs from blood to brain. Such a standard method has been developed based on observations that for many drugs, including those used in this study, drug concentration in cerebrospinal fluid approximates that in brain. Measurement of this transfer during the attainment of a steady state permits the estimation of a rate constant and an equilibrium constant. Anesthetized dogs were the test animals. Constant plasma concentration of drugs was maintained for 3 or more hr. Samples of cerebrospinal fluid were obtained through a plastic catheter in the cisterna magna. The ratio of drug concentration between cerebrospinal fluid and plasma water was plotted against time and both the final ratio (an equilibrium constant) and the time to reach one half this ratio (a rate constant) were estimated. After antipyrine or sulfanilamide administration for a 3-hr period, drug concentration in cerebrospinal fluid closely approximated that in plasma water, while with sulfanilic acid, the concentration in cerebrospinal fluid did not exceed 5% of the plasma water concentration. The half-time for antipyrine was about 10 min and that for sulfanilamide was about 80 min. The use of these substances with different constants permits the construction of standards for plasma-cerebrospinal fluid transfer. It is possible to employ these standards to study the behavior of other drugs, as well as the functions of the plasma-cerebrospinal fluid barrier under physiologically altered states.

1409. EFFECT OF PURINE RIBOSIDE ON POTASSIUM FLUX OF STORED HUMAN ERYTHROCYTES. Searle B. Rees, Thomas J. McManus, Fabian J. Lionetti, and John G. Gibson, 2nd. (intr. by Dale G. Friend). Depts. of Medicine, Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.

Optimum accumulation of cell potassium lost during cold storage requires collection and preservation in an isotonic, pH 7.3, phosphate, dextrose, heparin medium. Eighteen mM of adenosine and 27 mM of glucose were added per liter of erythrocytes, and cells were resuspended in a phosphate-bicarbonate buffered aliquot of fresh plasma for equilibration at 37°C. Glucose alone provided maximum net potassium accumulation of 2.5-3.0 mEq/l. rbc x hr during the 1st hr of equilibration of cells stored 6-8 days at 5°C. Net potassium uptake with dextrose and no added adenosine was observed for cells stored no longer than 2 wk. After the 1st wk of storage addition of purine riboside significantly enhanced net potassium accumulation and during the 3rd and 4th wk proved to be essential for net potassium uptake. Flux studies suggest that net potassium accumulation in vitro is dependent on an accelerated efflux of sodium exceeding the stimulated potassium influx of 4.5-5.5 mEq/l. rbc x hr. This leads to a compensatory net loss of cell water. Despite the lowering of cell potassium by cold storage, passive efflux is not retarded during 37°C equilibration due to a gradual increase in the passive potassium diffusion constant as a function of storage time. The energy and intermediates derived from nucleoside phosphorolysis do not increase the erythrocyte's resistance to passive flux until cells have deteriorated markedly with 4 wk of storage at 5°C.

1410. BIOCHEMICAL MECHANISM OF ACETALDEHYDE UNCOUPLING OF OXIDATIVE PHOSPHORYLATION. M. Joseph Rehak* and Edward B. Truitt, Jr., Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore.

The uncoupling of oxidative phosphorylation from respiration by acetaldehyde (AcH) in rat brain mitochondria has been reported from this laboratory (Truitt et al. Quart. J. Stud. Alcohol In press). This action, produced by concentrations of AcH occurring normally and in the Antabuse - alcohol reaction, is not shared by alcohol in this in vitro system. The uncoupling action of AcH is not mediated by a depressant effect on the hexokinase-glucose "trapping system." It is not sensitive to reversal by excess Mg^{++} as for tetracycline antibiotics and thyroxin. AcH does not produce a stimulation of respiration by brain mitochondria in the phosphate acceptor deficient system as does dinitrophenol. With equal AcH concentrations brain mitochondria exhibit greater sensitivity to the uncoupling action than does rat liver mitochondria. This was shown not to be explainable on the basis of the higher rate of AcH metabolism by liver mitochondria. Antabuse, final concentration 0.5 mM (dissolved in polyethylene glycol 200 and added to flask with final PEG concentration 1.9%) produced a decided uncoupling of oxidative phosphorylation in rat brain mitochondria, but not with liver mitochondria. The effect of combined acetaldehyde and Antabuse in uncoupling oxidative phosphorylation is more striking in the liver where neither agent has a significant action alone. The significance of these results will be discussed in relation to theory of the interaction of alcohol, Antabuse and acetaldehyde. (Supported by Grant B-1075, Natl. Insts. of Health, PHS.)

1411. DETERMINATION OF CATECHOL AMINES IN THE PLASMA OF PSYCHIATRIC PATIENTS. Joseph Reilly and Peter F. Regan III.* Depts. of Psychiatry and Pharmacology, Cornell Univ. Med. College and New York Hosp., New York City.

The catechol amine content in the plasma of 75 psychiatric patients and 30 normal subjects was determined. Assays were performed by means of modifications of the chemical methods described by Weil-Malherbe and Bone and by Lund. With the Weil-Malherbe method, the average value for plasma epinephrine for all subjects was 0.75 μg/l. and for plasma norepinephrine was 2.1 μg/l. With the Lund method, the average value for plasma epinephrine was 0.16 μg/l. and for plasma norepinephrine was 0.34 μg/l. With a single determination, it is impossible to correlate plasma epinephrine and norepinephrine values with the diagnostic category or the emotional state of patients. Furthermore, it is not possible to distinguish

between the plasma levels of epinephrine and norepinephrine maintained by patients, and the levels maintained by controls. Initial observations, however, indicate that plasma epinephrine and norepinephrine fluctate in conjunction with the changing emotional state in the individual patient. It is known that inherent difficulties with the Weil-Malherbe and the Lund methods produce distortion in the results; the use of repeated tests in the individual patient allows for control of these difficulties.

1412. RING CLOSURE AND FUNGISTATIC ACTIVITY. Alfred A. Renzi, Anna C. Garner and Alfred Burger (intr. by Lawrence J. Milch). Dept. of Pharmacology-Biochemistry, School of Aviation Medicine, USAF, Randolph AFB, Tex., and Dept. of Chemistry, Univ. of Virginia, Charlottesville.

The fungistatic activity of substituted phenothiazines against certain dermatophytes in vitro has been reported by several investigators (Mitchell et al. J. Am. Pharm. A. (Scient. Ed.) 41:472, 1952). Such empirical screening studies, however, usually point out the variations in activity due to presence, absence, or change in ring substituents rather than any effects due to an alteration of the ring structure itself. Exploration of this latter possibility was accomplished in our laboratories by testing 16 substituted phenothiazines available from commercial sources along with 25 additional compounds synthesized in our laboratories to be either very similar or identical to the phenothiazines except that the N-C linkage at the 10-11 position was open. Evaluation of all drugs was done by the filter paper disc technic on inoculated Sabouraud agar plates. Drugs were all screened at a 0.01 M concentration and in those instances where complete inhibition was observed, retested at 0.001 M and 0.0001 M concentrations. A grading scale was devised whereby each compound achieved an index based on the minimum concentration required for complete inhibition of each fungus. The grading scale was validated by simultaneously evaluating hydrocarbon isoquinolinium derivatives that have been shown to be extraordinarily effective against the same series of dermatophytes in vitro. The results indicated that cleavage of the N-C bond at the 10-11 position in the phenothiazine nucleus enhanced fungistatic activity and, at the same time, reduced the requirement for chain length in the secondary amine ordinarily attached to the nitrogen in the center ring.

1413. RELEASE OF NOREPINEPHRINE FROM THE ISOLATED HEART. J. A. Richardson and E. F. Woods.* Dept. of Pharmacology, Med. College of South Carolina, Charleston.

Previous investigators have shown that, under certain conditions, acetylcholine may produce epinephrine-like effects on the heart (J. Physiol. 104: 392, 1946). In the present investigation, epinephrine and norepinephrine concentrations in perfusates from isolated rabbit hearts were determined by a fluorimetric method described elsewhere (J. Lab. & Clin. Med. 47:832, 1956). The hearts were perfused in a modified Langendorff preparation with oxygenated Ringer-Locke solution containing sufficient atropine to abolish the inhibiting action of acetylcholine. Large doses of acetylcholine (25-200 μg) increased heart rate and amplitude of contraction and correspondingly produced substantial increments in the concentration of norepinephrine in the perfusion fluid. Epinephrine values remained essentially unchanged. Bioassays of the heart perfusates were in good agreement with the chemicall determined values. Perfusates collected during acetylcholine stimulation produced increments in arterial pressure and heart contractile force, as measured directly with the strain gauge arch, in acutely prepared dogs, and inhibited the contraction of isolated rabbit intestinal segments.

1414. EFFECT OF CHLORPROMAZINE ON PHYSICAL PROPERTIES OF BILE. J. Alfred Rider, Maureen Meikle, Lourdes F. Agcaoili and Jere Deroin (intr. by Kenneth P. DuBois). Dept. of Medicine, Univ. of California, San Francisco.

The pathogenesis of obstructive jaundice associated with ingestion of chlorpromazine is not understood. Change in physical properties of bile could be a factor involved. Specific gravity, fiscosity, bilirubin and total volume in 24-hr specimens were determined on 2 human subjects with indwelling biliary T-tubes, and on 14 adult rats of the Long-Evans strain, whose specimens were collected via a polyethylene common duct tube. Nine experimental animals were given 10 mg/kg of chlorpromazine s.c. daily for 42 days, but accurate data was only available on 5 drug-treated and 4 of 5 control animals. In the first patient given 15 mg 4 times daily i.m. for 48 hr average values before chlorpromazine administration were: specific gravity 1.00975, viscosity 1.1147 centipoise, bilirubin 39.55 mg %, total volume 275 cc; during therapy: specific gravity 1.0089, viscosity 1.1209, bilirubin 38.15, total volume 335; and after therapy: specific gravity 1.0100, viscosity 1.1167, bilirubin 30.56, total volume 309. In the second patient given 50 mg 4 times daily orally for 28 days the values before therapy were: specific gravity 1.0074, viscosity, 1.1576, bilirubin 41.1, total volume 430; during therapy: specific gravity 1.00727, viscosity 1.1538, bilirubin 33.1, total volume 574.5; after therapy: specific gravity 1.0075, viscosity 1.2261, bilirubin 26.25, total volume 527.5. In both cases bilirubin fell after therapy and volume increased. The average values for control rats were: specific gravity 1.00925, viscosity 1.2252, bilirubin 4.02, total volume 20.5. Experimental rats exhibited the following average values: specific gravity 1.00886, viscosity 1.2112, bilirubin 4.60, total volume 15.84. There was a decrease in volume and increase in bilirubin in the experimental animals. These effects were opposite to those in humans. Microscopic sections of rat livers were negative.

1415. MEASUREMENT OF SPONTANEOUS ACTIVITY IN MEDULLATED AND NONMEDULLATED NERVE FIBERS. J. M. Ritchie* and W. W. Douglas. Natl. Inst. for Med. Research, London, England and Pharmacology Dpt., Albert Einstein College of Medicine, New York City.

During a study on the nonmedullated vagal afferents a technique was developed whereby the spontaneous activity in medullated and nonmedullated fibers of a whole nerve trunk could be identified and measured. The method relies on the fact that the more spontaneous activity there is in a nerve the more difficult it is for evoked antidromic impulses to propagate any distance because they are the more liable to be extinguished by prodromic impulses coming from a sensory terminal - or a motor neurone. Thus, in a nerve containing two or more distinct groups of fibers, such as the cervical vagus or cervical sympathetic trunks, it has been shown that spontaneous activity in any particular group of fibers is reflected in a reduction in size of the corresponding component of the compound action potential (compared with its size in the inactive nerve). Furthermore, from the results obtained with this technique it has been

possible to calculate the average frequency of firing of an active group of fibers: for example, it was estimated that the nonmedullated fibers of the cervical sympathetic trunk fire at a rate of IO impulses/sec in the terminal stages of asphyxia. The greatest virtue of the present technique lies in its ability to detect and measure activity in nonmedullated fibers in mixed nerves - which has not been possible hitherto.

1416. NUCLEIC ACID DERIVATIVES IN HEART MUSCLE. Jane S. Robb. Dept. of Pharmacology, State Univ. of New York, Upstate Med. Ctr., Syracuse.

Studies previously reported, have been extended to include possible effects of drugs on these derivatives. To individual dogs, acetylcholine, epinephrine, potassium chloride, veratridine, crystodigin, or ouabain, was administered in minimum lethal dosage. When a full effect had developed, the heart was excised, dropped into liquid N and when frozen was weighed, pulverized and homogenized in ice cold solution. Extraction of the entire heart (divided into atria, free wall of right ventricle and left ventricle plus septum) provided abundant material for duplicate analyses by paper chromatography as well as by use of resin columns. Compared to normal controls, the sum of the adenine derivatives (adenine, adenosine, AMP, ADP, ATP) was decreased by each drug, not more by a relaxant than by a contractant. By each method the sum of the inosine derivatives was increased. Smellie (The Nucleic Acids, vol. 2, p. 424) proposes that adenine by action of adenase is catabolized to hypoxanthine and that adenosine is catabolized to inosine and thence to hypoxanthine. This being true, one would postulate that as adenine and adenosine decrease the sum of inosine plus hypoxanthine should increase. This proves to be the case. However, the increase due to inosine for hypoxanthine did not increase with all drugs, in fact decreased with acetylcholine and veratridine. We do not find an increase in uracil (as per fig. 6 of Smellie) but some extracts do show thymine, which Dorough and Seaton (J. Am. Chem. Soc. 16:2875, 1954) did not find in normal mouse hearts. In no series have we found appreciable amounts of xanthine. Our data re resins differ from that of Khairallah and Mommaerts (Circulation Res. I:8, 1953) in having less ATP and ADP in both relaxed and contracted hearts, than they reported. In all hearts, except that treated with KCl, a third component was eluted by 0.003 N HCl. We find adenosine, adenine, AMP and IMP throughout the series.

1417. TOXICITY AND PHARMACOLOGIC PROPERTIES OF NOFORMICIN. Harry J. Robinson, Otto E. Graessle,* Robert B. Stebbins* and Henry Siegel.* Merck Inst. for Therapeutic Research, Rahway, N. J. and Pathology Dept., Albert Einstein College of Medicine, New York City.

Noformicin, a substance produced by Nocardia formica has been reported active against virus infections of both plants and animals. In influenza infected mice, noformicin has been reported to be efficacious when administered orally, s.c., i.v. or as a nebulized spray. In mice and rats, mortality following parenteral administration of single doses of crystalline noformicin is usually delayed except when extremely high doses are given. Acute deaths are preceded by lethargy and diarrhea; late deaths by body weight loss, alopecia, tremors, and partial paralysis. The respective LD_{50}'s in mice following single oral, s.c. and i.v. doses are 215, 55 and 50 mg/kg. Rats are more susceptible than mice to the acute effects of noformicin. When administered to rats by repeated s.c. injection for 30 days noformicin in doses of 0.63 and 1.25 mg/kg depressed weight gain; higher doses produced a precipitous weight loss, nasal bleeding, paralysis of the hind limbs and death after 4 days. At necropsy, these animals showed foci of consolidation in the lungs, pale livers, congestion of the gastrointestinal tract and evidence of irritation at the site of injection. The pharmacologic properties of the crystalline substance will be discussed.

1418. GASTRIC JUICE STUDIES IN RATS. Harry Rosen,* Albert Blumenthal,* Mary H. Nead,* Richard Tislow and Joseph Seifter. Wyeth Inst. for Med. Research, Radnor, Pa.

Alumina gel; sodium polyanhydromannuronic acid sulfate; alpha, alpha'-diphenyl-gamma-dimethylaminobutyramide ethobromide (Wy-985); 10-(3-dimethylaminopropyl)-phenothiazine hydrochloride (Wy-1094); 10-(3-dimethylaminopropyl)-2-acetylphenothiazine maleate (Wy-1172) and various combinations of these were studied for their effects on gastric secretion in rats. Fasted pylorusligated rats, with a 4-hr secretion period were utilized in these experiments. Determinations were made for gastric juice volume, pH, free acid, total acid and pepsin. Alumina gel raised pH and reduced free acid and pepsin. Sodium polyanhydromannuronic acid sulfate reduced pepsin while raising free acid concentration. Wy-985 reduced gastric juice volume and free acid. Wy-1094 and Wy-1172 both reduced gastric juice volume and produced some lowering of free acid concentration. Of the combinations used that of alumina gel, sodium polyanhydromannuronic acid sulfate, Wy-985 and Wy-1172 was the most efficient. It reduced gastric juice volume, raised pH, inhibited free acid and lowered pepsin concentration.

1419. BIOSYNTHESIS OF NOREPINEPHRINE AND EPINEPHRINE BY THE ISOLATED, PERFUSED CALF ADRENAL. George Rosenfeld,* Lemuel C. Leeper* and Sidney Udenfriend. Naval Med. Research Inst. and Natl. Heart Inst., Bethesda, Md.

Based mainly on studies in intact animals, it has been shown that tyrosine is the precursor of norepinephrine and epinephrine and that dihydroxyphenylalanine (DOPA) and dihydroxyphenylethylamine (DOPE) are intermediates in their biogenessi. However, such studies do not reveal which biosynthetic reactions occur in the adrenal gland itself. While the use of adrenal homogenates has been informative in this regard, many questions concerning the biosynthesis of catechol amines by the adrenal remain unanswered. In the present studies, their formation was investigated under relatively simple and defined experimental conditions obtained by perfusing intact calf adrenals, isolated from the metabolic influences of other tissues, with an artificial medium containing various C^{14}-labeled substrates. The conversion of tyrosine and DOPE to norepinephrine was demnnstrated by this technique. The formation of epinephrine from DOPE and norepinephrine was also observed, provided a methyl donor was added to the perfusion medium. Methionine was found to be the most efficient methyl donor while formate and formaldehyde exhibited only slight activity; choline was completely inactive. Demethylation of epinephrine to norepinephrine did not occur. The conversion of DOPE to norepinephrine was markedly suppressed (90%) in glands which were perfused anaerobically with 2, 3,5-triphenyltetrazolium chloride affording them an active oxidative

metabolism. The perfusion technique employed herein yielded relatively large amounts of labeled norepinephrine and epinephrine despite the low activities of the enzyme systems participating in their biosynthesis.

1420. ETHANOL FRACTIONATION OF CHICK EMBRYO GROWTH FACTOR. Morris Rosenfeld. Dept. of Pharmacology and Expt. Therapeutics, Johns Hopkins Univ. School of Medicine, Baltimore, Md.

Chick embryo extract has been subjected to low-temperature alcohol fractionation following the general procedures of E. J. Cohn. Twelve-day embryos were homogenized with an equal volume of 0.15 M NaCl in a Waring Blendor at 0° to +5°C. The preparation was centrifuged and the pH of the supernatant was adjusted to 7.4 with 1.0 M Na_2HPO_4, with the solution immersed in a 0° bath. A precipitate with growth-promoting activity was obtained by the dropwise addition of cold 80% ethanol to bring the final alcohol concentration to 20%. The pH fell to 6.8 during the precipitation which was carried out with constant stirring in a -5° bath. A 2nd precipitate with considerably less activity came down when the pH of the supernatant was reduced to 4.8 by the addition of 1.0 M, pH 4.0, acetate buffer. A 3rd precipitate, obtained by increasing the ethanol to 40%, showed little or no activity. Each of these precipitates was taken up in half the original volume of 0.15 M NaCl and lyophilized. The powder was dissolved in Gey's solution, modified by omitting NaCl. Some insoluble residue was removed by 1/2 hr of centrifugation at 24,000 g and at a temperature of 0° to +5°. Sterilization was accomplished by passing the reconstituted solution through a series of Selas microporous filters. Growth assays were conducted in roller-tube cultures of 12-14 day embryo chick heart. (Supported by a research grant, C-1841, from the Natl. Cancer Inst., PHS and by a grant-in-aid, C-6, from the American Cancer Soc.)

1421. ANTITHYROID AGENTS IN EXPERIMENTAL ARTERIOSCLEROSIS. A. P. Roszkowski* and Y. T. Oester. Dept. of Pharmacology and Experimental Therapeutics, Stritch School of Medicine of Loyola Univ., Chicago, Ill.

Previously, Roszkowski and Oester (Fed. Proc. 15: No. 1, 1956) demonstrated that thyroidectomy in rabbits completely prevents the production of an experimental arteriopathy induced by daily i.v. injections of epinephrine. To further explore this finding, several different methods were used in an attempt to promote an athyroid or hypothyroid state in rabbits. This was then followed by the routine epinephrine sclerogenic regimen. A group of 15 rabbits (controls) was given 0.5 mg/kg daily i.v. injections of epinephrine for a total of 15 days. An experimental group of 10 rabbits received the above treatment plus daily oral administrations of propylthiouracil, 125 mg/kg. (Forty-six percent) of the animals receiving the i.v. epinephrine regimen displayed aortic lesions. This is a general agreement with previous findings (Friedman et al. Arch. int. Pharmacodyn. 102:226, 1956). In contrast, all animals treated with epinephrine and propylthiouracil displayed aortic lesions. Microscopic examination of the aortae from both groups revealed that the lesions were all of the medial type. Intimal involvement was minimal or generally absent. Microscopic examination of the thyroid gland from those animals receiving propylthiouracil revealed the tissue to be normal; especially, no evidence of columnar thyroid epithelium. The high incidence of medial sclerosis in the group receiving propylthiouracil indicates that propylthiouracil augments epinephrine arteriosclerosis. Another 2 groups of animals were treated without epinephrine. Ten rabbits in each group were given oral administrations of 125 mg/kg of propylthiouracil and thiouracil, respectively, for a total of 15 days; 50% of the propylthiouracil animals showed aortic lesions; 40% of the thiouracil animals displayed aortic lesions. Microscopic examination of these aortae showed that undulations, necrosis and hyalinization of the elastic components of the media occurred. Examinations of the thyroid glands also demonstrated "normal" state. It is concluded that the antithyroid agents, propylthiouracil and thiouracil, exert a sclerogenic influence upon the rabbit aorta. It is unlikely that this occurs by way of the thyroid mechanism.

1422. PHOSPHOLIPID STRUCTURE AND PARTIAL THROMBOPLASTIC ACTIVITY. George Rouser, Sidney D. White and David Schloredt (intr. by Howard R. Bierman). Depts. of Biochemistry and Hematology, Med. Research Inst., City of Hope Med. Ctr, Duarte, Calif.

The nature of the phospholipid (PL) capable of decreasing clotting time of recalcified, citrated, platelet-poor plasma has been re-evaluated. Highly purified PL and natural mixtures have been studied after chromatographic examination for water soluble and lipid constituents. Lecithin, sphingomyelin, cerebron, glycerides, cholesterol and most water soluble PL hydrolysis products are inactive and ethanolamine is inhibitory. Phosphatidyl ethanolamine (PE) or phasphatidic acid (PA) isolated chromatographically greatly decrease clotting time whereas higher fatty acids have only a small effect. Phosphatidyl serine and monophosphoionsitide (McKibben. J. Biol. Chem. 220:537, 1956) inhibit. PE is the natural active PL from tissues (PA not present). PE content of PL mixtures parallels shortening of clotting time at 1-5 mg % PL: platelets > brain > soybean > granulocytes > lymphocytes > plasma. PL mixtures treated with ninhydrin lose 98-99% of their activity. Activity depends upon the state of dispersibility in water; saturated PE is inactive (poor water emulsification), while natural unsaturated PE (good aqueous emulsion) is highly active. Hydroxylation by performic acid of unsaturated PE reduces activity over 99% (PE water solubility increased). The following working hypothesis is formulated: PE or PA reacts with Ac^{2+} and a negatively charged site (perhaps on a protein) to form an active colloidal complex. Thromboplastic inhibitors prevent formation of the active complex by reacting with one or more of the components of the complex. High molecular weight acidic or basic substances inhibit, while non-ionic, or neutral ionic, substances are inactive.

1423. EFFECT OF CERTAIN CARCINOGENIC AGENTS ON THE TOTAL NON-PROTEIN SULFHYDRYL CONTENT OF THE RAT LIVER. Pierre-Georges Roy,* Tom S. Miya and C. Jelleff Carr. Dept. of Pharmacology, Purdue Univ., Lafayette, Ind.

Early reports suggested the interaction of carcinogens with SH groups of cell proteins and particularly of enzyme proteins. Evidence is increasing that carcinogenic agents must in some way interfere with cell proteins in order to exert their cytochemical effects. The carcinogenic influence of p-Dimethylaminoazobenzene (DAB) and β-Naphthylamine (BNA) in inducing rat liver tumor and the total nonprotein sulfhydryl (TNPSH) content has been studied.

Sprague-Dawley female rats were maintained on a shock diet and a low protein (8%)-low riboflavin diet (0.1 gm/100 lb diet). In each instance, the carcinogens were incorporated thoroughly in the food; 600 mg of DAB or 100 mg of BNA per 1 kg of diet. Control animals were fed the same diets without the carcinogens. The feeding period of the deficient diet was 16 wk and the normal diet 24 wk. Five animals of each control and experimental group were killed every 4 wk and the TNPSH content of the liver determined amperometrically. The incorporation of DAB and BNA in the diet increased the TNPSH concentration of the rat liver. The addition of BNA to a low protein-low riboflavin diet caused a maximum decrease after 4 wk, whereas DAB, under the same experimental conditions, induced a pronounced fall after 8 wk, attaining its lowest value at the 16th wk. The low protein-low riboflavin treated control animals showed a decrease after 12 wk, but liver TNPSH values returned to near normal in 16 wk. These carcinogenic agents, when added to the stock diet, also decreased the liver sulfhydryl concentration. Here the maximum decrease was recorded after 12 wk of feeding with a gradual return to normal values at the 20th wk. The liver sulfhydryl content of the control rats remained relatively constant throughout the experiment. It is suggested that the low protein-low riboflavin diet creates a type of stress which manifests itself in a decrease in the liver TNPSH content. The addition of a carcinogen to this diet augments the decrease in liver TNPSH content. Likewise, DBA and BNA incorporated in a stock diet will induce a drop in the liver TNPSH content, but to a lesser degree than with the low protein diet. Later, adaptation occurs and the sulfhydryl level gradually rises to normal.

1424. A SPECTROPHOTOMETRIC METHOD FOR ASSAYING ANTICHOLINESTERASE ACTIVITY. A. A. Rubin, J. Mershon, M. E. Grelis and I. I. A. Tabachnick. (intr. by W. M. Govier). Dept. of Pharmacology, Research Div., Schering Corp., Bloomfield, N. J.

A recent report described a spectrophotometric assay whereby purified cholinesterases catalyze the hydrolysis of chiocholine esters (Biochim. et biophys. acta 21:580, 1956). The time course of this reaction is reflected directly by changes in optical density at 250 mμ as the thiocholine ester is hydrolyzed to thiocholine and acid. In the present study this procedure has been modified somewhat so that a rapid (5 min) and reproducible estimation of anticholinesterase activity may be obtained. All reactants were dissolved in 0.1 M sodium phosphate buffer, pH 6.4, pipetted into 1.0-cm cuvettes and placed in the appropriate temperature controlled area (thermospacers, 30°-31°C) of the Beckman DU spectrophotometer. The control sequence consisted of 1.0 ml buffer, 1.0 ml enzyme (40 U Winthrop-Stearns bovine red cell acetylcholinesterase or 1:500 dilution of Cholase, a human plasma cholinesterase) and 1.0 ml substrate (acetylthiocholine iodide or butyrylthiocholine iodide, final concentration, 6.7×10^{-4} M). The test sequence was similar except for the substitution of inhibitor for buffer. In those instances where the inhibitors absorbed strongly at 250 mμ, i.e. phenothiazine derivatives, anticholinesterase activity was determined by reducing the light path with the use of 0.5-cm cuvettes. Changes in optical density were plotted against time and the straight line relationships so obtained under converted into units according to the formula, $U = (-\Delta O.D. \times 1000)/\text{min}$. Percentage inhibition was determined by comparing control and test values.

1425. EVALUATION OF CHLOROTHIAZIDE, A SALURETIC-DIURETIC AGENT, IN THE DOG. Horace F. Russo,* John E. Baer,* Ronald M. Noll* and Karl H. Beyer. Merck Inst. for Therapeutic Research, West Point, Pa.

Although chlorothiazide (6-chloro-7-sulfamyl-1, 2,4-benzothiadiazine-1,1-dioxide) has been characterized as a saluretic agent (Beyer et al. Fed. Proc. 1957) to designate its ability to promote the excretion of chloride and sodium ions in the dog, it is a potent in vitro inhibitor of carbonic anhydrase. Experiments were designed to evaluate chlorothiazide in the dog, especially with reference to acetazolamide and meralluride. The intravenous administration of acetazolamide (2.5 mg/kg prime and an infusion of 3.0 mg/kg/hr) in clearance experiments caused an increase above the control values for sodium and potassium excretion and in urinary pH. When chlorothiazide in the same dose was superimposed in a 3rd phase, a further increase in cation excretion and profound chloruresis occurred with no further increase in pH. If additional acetazolamide, instead of chlorothiazide, was given there was only an exaggeration of the initial effect, with no chloruresis. Although the diuretic effects of meralluride are reversed by dimercaptopropanol (BAL), BAL has little effect on chlorothiazide-induced excretion of water or electrolytes. Both meralluride and chlorothiazide were shown to counteract the antidiuretic action of vasopressin (Pitressin). In clearance studies on adrenalectomized dogs chlorothiazide reversed the sodium retention caused by cortisone, hydrocortisone and 9α-fluorohydrocortisone. In long-term experiments, wherein salt and fluid retention was produced by the daily administration of steroids to normal dogs, oral or parenteral chlorothiazide caused a prompt profound diuressis with the reduction of weight to normal within 24-48 hr. Withdrawal of a daily maintenance dose (5 mg/kg) of chlorothiazide during steroid administration permitted weight gain to recur promptly.

1426. PHTHALIMIDE COMPOUNDS IN ACUTE BARBITURATE POISONING. Lawrence F. Sancilio,* Theodore Koppanyi, Leonard M. Rice* and Charles F. Geschickter.* Pharmacology and Pathology Depts., Georgetown Univ. Schools of Medicine and Dentistry, Washington, D. C.

A series of phthalimides, including 1,4-endoxy-hexahydrophthalimide and other related compounds, were administered intravenously in doses of 20-100 mg/kg to rats, rabbits and dogs. None of the compounds produced central excitement or convulsions. Following the intraperitoneal administration of 60 mg of pentobarbital sodium/kg to rats, however, they shortened the sleeping time from 50 to 100%. Rabbits, given 25 mg of pentobarbital sodium/kg, i.v., may be instantly awakened by the administration of 100 mg/kg of these compounds. Dogs, given 30 and 35 mg/kg of pentobarbital sodium, showed an awakening effect in about 30 min following administration of 100 mg/kg of the phthalimides. Dogs anesthetized with urethane (2gm/kg) also showed the denarcotizing effects of graded doses of 1,4-endoxy-hexahydrophthalimide. Preliminary experiments revaled life-saving effects of 1,4-endoxy-hexahydrophthalimide in rabbits poisoned by LD_{90} of sodium pentobarbital. Although respiratory stimulation upno injection of these compounds was not seen in normal animals, it was pronounced in barbiturate-treated animals. It appears that these compounds are not analeptics in the usual sense of the word, namely their denarcotizing and life-saving dosage does not produce obvious

CNS excitation accompanied by seizures. (Supported by the Charles F. Geschickter Fund.)

1427. ABSORPTION OF DRUGS BY THE HUMAN STOMACH. Lewis S. Schanker and C. Adrian M. Hogben (intr. by Bernard B. Brodie). Natl. Heart Inst., Bethesda, Md.

Previous communications from this laboratory have described absorption of drugs by the rat stomach and secretion of parenterally administered drugs by the dog stomach. The results were consistent with the hypothesis that the gastric mucosa has the properties of a lipoid barrier, allowing rapid transfer of the unionized moieties of organic electrolytes while impeding the transfer of the ionized moieties. The present report describes studies on the absorption of drugs by the human stomach. Solutions of various drugs were administered via Levin tubes to fasted normal subjects in the left supine position. Incorporation of phenol red in the solutions permitted estimation of the initial and final volumes and the correction for the concentration change due to dilution. The decrease in concentration of drug was determined at 10-min intervals for 40 min. Acidic drugs, such as aspirin and secobarbital, were rapidly absorbed. Moderately strong bases, such as quinine and ephedrine, were poorly absorbed, while the very weak base, antipyrine, was fairly rapidly absorbed. These results, which parallel the previous study of absorption by the rat stomach, demonstrate that the ionization constant and the acidic or basic nature of a drug are important in determining gastric absorption. The human stomach is potentially an important site of absorption for many orally administered drugs if they are sufficiently soluble.

1428. HISTOCHEMICAL DETERMINATIONS OF GLYCERALDEHYDE PHOSPHATE AND GLYCEROPHOSPHATE DEHYDROGENASES IN BRAIN. Robert T. Schimke (intr. by Oliver H. Lowry). Dept. of Pharmacology, Washington Univ., St. Louis, Mo. and Dept. of Pharmacology, Stanford Univ., Stanford, Calif.

The Fluorescent properties of DPNH and DPN were utilized to determine enzymatic activity in as little as 0.05 μg of frozen-dried brain. Studies on the rate of reduction of DPN and oxidation of DPNH by rabbit brain homogenate in the presence of tricose phosphate prepared enzymatically from hexose diphosphate included evaluations of K_m for substrate and cofactor, pH optimum, Q_{10}, enzyme stability and presence of interfering enzymes or inhibitors. Frozen-dried section of rabbit cerebellum and Ammon's horn and monkey (M. mulatta) retina were dissected to obtain samples which were relatively homogeneous as to tissue component. Rabbit brain homogenate glyceraldehyde phosphate dehydrogenase (GAPD) and glycerophosphate dehydrogenase (GOPD) activities were 10.4 and 0.84 moles DPN(H)/kg dry wt/hr (MKH). GAPD in layered structures of rabbit brain studied were: myelinated fibers, 1.14 MKH; dendrites, 5.50 MKH; cell bodies of neurons, 8.32 MKH; neuropile, 5.94 MKH. Results from Ammon's horn and cerebellum were similar for comparable tissue components. GOPD in Ammon's horn was found to be: myelinated fibers, 1.25 MKH; dendrites 1.50 MKH; neuron cell bodies, 1.17 MKH; and neuropile 1.00 MKH. GOPD in molecular, granular and white matter layers of cerebellum were 0.70, 0.66 and 0.77 MKH respectively. GAPD in monkey retina was found to be markedly different in certain layers. The outer plexiform layer had the highest activity, 20.2 MKH; this was twice that of the adjacent two layers. The outer rod and cone segment had the lowest activity, 0.87 MKH. GOPD in monkey retina ranged from 0.27 MKH in the outer rod and cone segment to 1.53 MKH in the inner plexiform layer. As in Ammon's horn in the rabbit, retina GOPD was relatively high in layers containing large amounts of lipid.

1429. PHARMACOLOGICAL EFFECTS OF CERTAIN THIAZOLONES WITH SPECIAL REFERENCE TO THEIR ANALGETIC PROPERTIES. J. A. Schneider, E. B. Sigg,* M. McArthur* and G. de Stevens.* Research Dept., Ciba Pharmaceutical Products Inc., Summit, N. J.

A series of thiazolones produced a surprising reduction in responsiveness to painful stimuli in both mice and rats when given by various routes of administration. Quantitative analysis of this potential analgetic effect was carried out by a method described by Gross (Helvet. Physiol. acta 5:C31, 1947) using the prolongation of reaction time to a painful heat stimulus as a measurement for pain sensation in mice. The most active compound of the series was N-methyl cyclopentano-[d]-4 thiazolin-2-one (Su-4027). Following subcutaneous injection the maximal analgetic effect was observed within 15 min which indicates a faster onset of action than either aminopyrine or morphine in a comparable dose range. Threshold effects for analgesia were obtained with this method in the range of 50-75 mg/kg s.c. Following 100 mg/kg s.c. more than 50% of the mice showed significant analgesia (Ipsen. acta pharmacol. et toxical. 5:321, 1949). Three hours following injection the analgetic effect was still present. The acute subcutaneous LD_{50} in mice was greater than 500 mg/kg. The material was also shown to be orally active. When compared to aminopyrine on a mg/mg basis for analgetic potency in the same experimental setup, Su-4027 was more potent, longer acting and less toxic. On the other hand, Su-4027 lacked antipyretic activity. It showed, however, definite antagonism to electroconvulsive shock in mice without providing significant protection against metrazol shock. Local anesthetic properties were absent. When tested for reflex activity in chloralose anesthetized cats only a transient enhancement of both monosynaptic and polysynaptic reflexes was observed. No effect on direct neuromuscular transmission could be demonstrated.

1430. DEPRESSOR RESPONSE PRODUCED BY EPINEPHRINE ADMINISTRATION TO THE CROSS-CIRCULATED HEAD. Richard H. Schneider, Lloyd Beck and David F. Bohr (intr. by M. H. Seevers). Depts. of Physiology and Pharmacology, Univ. of Michigan, Ann Arbor.

Taylor and Page (Circulation 4:563, 1956) ascribed physiological significance to a "cerebral buffer mechanism" revealed by a depressor response in the body of a dog when epinephrine was injected into the head. The head in their preparation was perfused by blood from a donor and connected to the body only by the spinal cord. The known baroreceptors were denervated and the response shown to be mediated by the sympathetic nervous system. Our studies were made with a similar preparation except for morphine-urethane anesthesia instead of pentobarbital and vascular separation at C_2 rather than between C_4 and C_5. With intact carotid sinuses and vagi, increasing perfusion pressure by means of a pump caused as much as 90 mm Hg fall in body blood pressure. When the carotid sinuses were denervated mechanically or by application of 10% cocaine, varying the perfusion pressure caused no variation in body blood pressure. Intra-arterial injection of epinephrine in the head then

caused a depressor response in the recipient body, varying between 0 and 50 mm Hg, and accompanied by a bradycardia. The depressor response was variably potentiated by cocaine applied to the sinus region or injected intravenously into the donor. Sectioning the vagi abolished almost entirely the depressor response. Thus in our preparations injection of epinephrine into the head caused a variable depressor response in the body primarily mediated by the vagus nerves, but with a minor component mediated by the spinal cord. (Supported by a grant from the Life Insurance Med. Research Fund.)

1431. ALTERATION OF IODINE METABOLISM BY FRACTIONS OF TUMOR. Kenneth G. Scott, Marie B. Daniels* and Ronald R. Scheline.* Dept. of Radiology, Univ. of California, San Francisco.

Earlier studies have shown that I^{131} metabolism is altered in rats and mice bearing transmissible or spontaneous tumors. This is characterized by "iodide trapping," i.e. reduced I^{131} excretion and thyroid hormone production and an increased I^{131} uptake in tissue. "Iodide trapping" is first observed in the tissue adjacent to a tumor, then in all of the tissues as the tumor growth proceeds. This is not observed in tumor homografts destined to regress because of strain incompatibility. An agent called "trap," which is capable of duplicating the syndrome described above when administered to normal animals, can be isolated from the polypeptide fraction of tumors. It is soluble in water, acetone and chloroform and is unstable in the purified state. The relationship of trap to tumor growth makes its complete identification desirable in the hope of developing blocking agents. A blocking agent, "antitrap," has been recovered from tumors. This agent appears to be a normal constituent of rat and beef liver and has been obtained from the albumin fraction of these tissues. It can be separated from the albumin by treatment with acetone. It is insoluble in chloroform and petroleum ether and displaces the iodide spot on paper chromatograms.

1432. PREPARATION AND ACTIVATION OF HUMAN PLASMINOGEN. J. T. Sgouris, K. B. McCall and J. K. Inman (intr. by C. W. Muehlberger). Div. of Laboratories, Michigan Dept. of Health, Lansing.

Seven lots of dried Fraction III have been fractionated by the procedure of Kline (J. Biol. Chem. 204: 949, 1953) and the potency of the final preparations was found to vary from 12.6-26.6 proteolytic units (PU)/mg N by the caseinolytic assay of Remmert and Cohen (ibid. 181:431, 1949). These values are lower than the value of 60 PU/mg N reported by Kline. To determine if this was due to the use of aged Fraction III, a dried Fraction III was prepared from relatively fresh, frozen Fraction II+III w paste. Upon subsequent fractionation this material yielded plasminogen with a potency of 35.5 PU/mg N. Dried Fraction III-3 was also investigated as a source of plasminogen. It was prepared by method 9 of Oncley et al. (J. Am. Chem. Soc. 71:541, 1949), with the omission of the final lysis step. This fraction proved to be an excellent starting material for the Kline procedure; the final product had a potency of 80-90 PU/mg N. Plasminogen prepared by the Kline procedure from dried Fraction III was heated for 10 hr at 60°C at pH 3.5 in order to destroy possible viral contaminants. Losses in activity of less than 5% were observed. The conversion of plasminogen to the active component (plasmin) was investigated by the following means: 1) spontaneous activation, 2) use of a placental activator, 3) streptokinase activation and 4) urokinase activation. Spontaneous activation by the method of Alkjaersig and Sherry did not occur with all preparations, even in the presence of small amounts of plasmin. The use of streptokinase has been considered undesirable because of its pyrogenic and antigenic properties. Human urokinase does not present these disadvantages. A potent non-pyrogenic urokinase free of direct proteolytic activity from human urine has been prepared by a method involving clarification, isoelectric precipitation and barium sulfate adsorption (129th Meeting, Am. Chem. Soc., April 1956). The activation of plasminogen by urokinase was studied at 0-+2°C in the absence of casein and at pH 7.4. This material has a relative potency of 1/17 that of streptokinase. These studies demonstrated the enzymatic nature of urokinase. Urokinase is labile below pH 5.0 with an optimal stability between pH 5.0 and 10.0. (Work done under an agreement between the Am. Natl. Red Cross and the Michigan Dept. of Health Labs.)

1433. SOME ANTISPASMODIC ACTIONS OF HYDROXYZINE. Theodore R. Sherrod and Gustavus A. Bobb.* Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.

Like many of the other tranquilizing agents, Hydroxyzine antagonizes the spasmogenic effects of Serotonin (5-hydroxytryptamine) in the isolated guinea pig ileum. This effect prompted experiments to determine the antispasmodic effect of Hydroxyzine against a variety of spasmogenic agents in both the isolated guinea pig ileum and the isolated guinea pig uterus. The isolated organs were maintained in an aerated Locke-Ringer solution at 37°C. The addition of Hydroxyzine in order to obtain a concentration in the bath of 1:100,000 or 1:200,000 completely counteracted the spasmogenic effects of Serotonin (5-hydroxytryptamine), 1:5 million; acetylcholine, 1:5 million; barium chloride, 1:500,000 and histamine, 1:5 million in both the guinea pig ileum and the guinea pig uterus. Concentrations of diphenhydramine comparable to those of hydroxyzine were effective against the spasmogenic actions of histamine and acetylcholine on the guinea pig ileum but considerably less effective against the spasmogenic actions of Serotonin and barium chloride on this structure. In the isolated guinea pig uterus Diphenhydramine was variably effective against the stimulating actions of barium chloride, acetylcholine, histamine and Serotonin, while Hydroxyzine invariably counteracted their spasmogenic effect.

1434. RELEASE OF BRAIN NOREPINEPHRINE BY RESERPINE. P. A. Shore, J. S. Olin* and B. B. Brodie. Lab. of Chem. Pharmacology, Natl. Heart Inst., Bethesda, Md.

Norepinephrine, measured fluorometrically, has been identified in rabbit brain by its activation and fluorescence spectra and by its rate of oxidation in buffers at various pH values. It is present in the highest concentration in the brain stem. Norepinephrine as well as serotonin is liberated in brain by the action of reserpine, confirming results reported by Holzbauer and Vogt (J. Neurochem. 1:8, 1956), who estimated the catechol amine by bioassay. The norepinephrine content of the brain stem, normally about 0.5 μg/gm, declined rapidly after the intravenous injection of 5 mg/kg reserpine. Within 4 hr the level declined by 80% or more. The low level persisted for many hours, increased slowly and attained the normal value only after a number of days. The implications of these findings in regard to a mechanism for the action of reserpine and the functions of norepinephrine

and serotonin in the central nervous system will be discussed.

1435. ACTION OF SYNCHRONIZING AND DESYNCHRONIZING DRUGS ON STRYCHNINE INDUCED CONVULSIVE CORTICAL ACTIVITY. P. Silvestrini, V. G. Longo and D. Vobet (intr. by K. R. Unna). Instituto Superiore di Sanita, Rome, Italy.

Strychnine was applied in dilute solution (0.05-0.1%) to the frontal cortex of unanesthetized noncurarized rabbits and of the rabbit "cerveau isole" preparation. The convulsive spikes recorded by surface electrodes on the frontal lobe were classified as liminal or supraliminal depending on whether or not they were absent during the desynchronization following acoustic stimuli (intact animal) and following electrical stimulation of the reticular formation ('cerveau isole"). Eserine given intravenously (0.05-0.1 mg/kg) caused EEG desynchronization together with blockade of the liminal spikes but had no effect on supraliminal activity. When applied topically (0.1%) eserine increased liminal and supraliminal spiking and caused the appearance of a "comb-shaped" repetitive discharge. Amphetamine given intravenously (1-2 mg/kg) desynchronized the tracing and blocked liminal spiking; however, it increased the frequency of supraliminal potentials. Applied locally it was ineffective. Scopolamine (0.05-0.1 mg/kg) synchronized the EEG, blocked the activation response to external stimuli and increased the frequency of both types of strychnine spiking. Topical application of scopolamine (1%) did not have these effects; it was, however, effective in blocking the repetitive discharge after combined application of strychnine and eserine. In the "cerveau isole" eserine caused desynchronization of the EEG and increased the frequency of liminal and supraliminal spikes. Although amphetamine desynchronized the EEG of this preparation, it did not affect strychnine spiking. Scopolamine had identical effects in "cerveau isole" and in the intact animal.

1436. CATECHOLAMINE BIOSYNTHESIS IN PATIENTS WITH PHEOCHROMOCYTOMA. Albert Sjoerdsma,* Lemuel C. Leeper* and Sidney Udenfriend. Natl. Heart Inst., Bethesda, Md.

The mechanisms for the biosynthesis of epinephrine and norepinephrine, as demonstrated in animals, have not been investigated in man due to the small amounts of these substances in blood and urine and the difficulty in obtaining fresh tissue for enzymatic studies. Patients with pheochromocytoma afford a satisfactory solution to these problems since they excrete large amounts of catecholamines and the tumors which are removed surgically provide a rich source of catecholamine-synthesizing tissue. Preliminary studies with C^{14} compounds in vivo indicate that both 3,4-dihydroxyphenylalanine (DOPA) and hydroxytyramine serve as effective precursors of norepinephrine and that the turnover of norepinephrine in these patients is quite rapid (half-life ca 7-9 hr) compared to the turnover previously obtained for norepinephrine and epinephrine in animal adrenals (half-life ca. 240 hr) (Udenfriend and Wyngaarden. Biochim. et biophys. acta 20:48, 1956). Homogenates and slices of pheochromocytomas have also been shown to convert hydroxytyramine to norepinephrine. The enzymes, monoamine oxidase and DOPA decarboxylase, were also demonstrated to be quite active in these tumors.

1437. BLOCKADE OF ADRENERGIC INHIBITORY RECEPTOR SITES BY 1-(3',4'-DICHLOROPHENYL)-2-ISOPROPYLAMINOETHANOL HYDROCHLORIDE. I. H. Slater and C. E. Powell.* Lilly Research Lab., Indianapolis, Ind.

Lilly Compound 20522 (1-(3',4'-Dichlorophenyl)-2-isopropylaminoethanol hydrochloride), the chloro-analogue of isoproterenol, in concentrations of 10^{-4} to 2×10^{-6} does not antagonize pilocarpine-induced construction of guinea pig tracheal chains, but does block epinephrine release of pilocarpine spasm. Injected into the femoral artery of an anesthetized dog, 20522 causea a transient increase in blood flow. A few minutes later, after the blood flow has returned to control level, the increase in flow induced by isoproterenol is reduced. On rabbit ileum a concentration which produced a slight decrease in tone antagonized the spasmolytic action of both epinephrine and isoproterenol. On the rat uterus, however, the compound caused a definite decrease in tone. In cats anesthetized with chloralose, 20522 may bring about a transient decrease in blood pressure. After the blood pressure has returned to normal, the pressor response to norepinephrine and carotid occlusion remains unchanged, the pressor response to epinephrine is enhanced and the secondary depressor phase, when present, is reduced. The depressor action of isoproterenol is reduced and, in an occasional animal, converted to a pressor response. The nictitating membrane contraction by sympathomimetic amines or by preganglionic stimulation is unchanged. Intraperitoneal injection of graded doses of 20522 failed to antagonize the lethal effect of 225 mg/kg i.v. of isoproterenol. The compound itself in mice increases the spontaneous activity qualitatively similar to that seen with amphetamine. 20522 does not seem to affect excitatory adrenergic sites. On the other hand, it seems probable that 25022 is combining with certain inhibitory adrenergic receptor sites without having much physiological effect and yet is competing for these sites with the physiologically active amines.

1438. EFFECTS OF ETHYL ALCOHOL ON THE COMPONENT MOVEMENTS OF HUMAN MOTIONS. K. U. Smith,* S. Harris* and F. E. Shideman. Depts. of Psychology, Pharmacology and Toxicology, Univ. of Wisconsin, Madison.

This investigation was undertaken to study the effects of small doses of alcohol on the component movements involved in walking, handwriting, manual assembly and manual placing motions, and is developmental in nature in applying special electronic methods of motion analysis to problems in the realm of psychopharmacology. Two dosage levels of alcohol and a placebo were employed. One dosage of alcohol was calculated to result in a maximal blood concentration of 0.06% and estimated to produce minimal behavioral effects, and the other was one-half this minimal level. A masking carrier of iced grapefruit fruie wasemployed for both drug and placebo administration. Each subject received the drug on different days according to a randomized design in which the individual served as his own control. Electronic techniques of motion analysis were designed especially for these experiments and were capable of measuring and summating separately the duration of the travel and manipulative movements in manual motions or that of the contact and step movements in gait. The duration of these basic components of motion were measured in relation to alcohol dosage levels in the tasks mentioned above. Analysis of the effects due to alcohol on the different component movements and task situations showed that different motor performances and movements are not affected equally by the drug. The contact

movements of the foot in gait and the contact movements of handwriting varied significantly in relation to alcohol level. The data are interpreted in relation to the application of electronic methods of motion analysis to appraisal of drug effects on man and infrahuman species.

1439. EVIDENCE THAT CHLORPROMAZINE EXERTS A GENERALIZED CENTRAL SYMPATHOLYTIC ACTION. Sydney Spector,* Donald F. Bogdanski* and Bernard B. Brodie. Lab. of Chem. Pharmacology, Natl. Heart Inst., Bethesda, Md.

The administration of chlorpromazine to dogs and rabbits lowers sympathetic activity as evidenced by hypotension, miosis, ptosis, relaxation of the nictitating membrane and hypothermia. The failure of chlorpromazine to block the cardiovascular effects of injected norepinephrine in dogs suggested the possibility that the lowering of sympathetic activity was largely central in origin. Further evidence of this was shown by the production of the aforementioned signs of inhibition by the intracisternal injection of doses of chlorpromazine which did not produce similar effects when administered systemically. These results suggest that chlorpromazine may exert its over-all effects on the autonomic system by antagonizing the sympathetic component of the central autonomic nervous system.

1440. SCREENING NEW COMPOUNDS: CRITERIA FOR ATARACTIC EFFECTS. Frederick Sperling. Sperling Labs., Arlington, Va.

In the course of a program of rapidly screening newly synthesized compounds for biological activity, criteria were established to distinguish ataractic from other central depressant effects based upon reports in the literature and on the actions of sodium bromide. These criteria included increased sleeping time following barbiturate administration; absence of effect on, or actual potentiation of, strychnine convulsions; skeletal muscular relaxation; and prolongation of the decrease in spontaneous activity following sodium bromide administration. Wistar-strain rats weighing 150-200 gm were used. All substances were administered intraperitoneally. Sodium bromide, 800 mg/kg, were followed 24 hr later by 40 mg/kg of secobarbital sodium. Sleep was prolonged from 68 min to 89 min. Reserpine, 1 mg/kg, increased barbiturate-induced sleep to 117 min and chlorpromazine, 20 mg/kg, to 133 min. Bromide alone reduced food intake slightly for 24 hr; reserpine alone showed no decrease, but chlorpromazine alone showed a large decrease in food intake. When the latter two were given 24 hr after bromide, only chlorpromazine showed a prolongation of the period of reduced food intake, reduction of spontaneous activity and muscular relaxation. Bromide resembled reserpine in having no noticeable effect on strychnine convulsions. Thus sodium bromide itself might be considered an ataractic agent. Two of the compounds in the screening program met all of the criteria including prolongation of the bromide effects.

1441. AEROBIC GLYCOLYSIS VALUES FOR HYPERTHYROID RAT BRAIN CORTEX SLICES. M. A. Spirtes and A. Andose.* Dept. of Pharmacology, Hahnemann Med. College, Philadelphia, Pa.

Attempts to show some metabolic change in vivo or in vitro in the mature brain during alterations of the thyroid state have usually failed. Thus we have shown for rats that oxygen consumption and anaerobic glycolysis of brain cortex slices remain unchanged during hyperthyroidism. Although recently Woodbury and co-workers have been able to show brain changes in cation distribution and in excitability for rats injected with thyroid hormone, they have not conclusively proven that these changes occur as a result of a direct effect on the periphery, since similar changes are seen after the injection of adrenal cortical hormones. The experiments described below also failed to show any change in aerobic glycolysis in brain cortex slices from hyperthyroid rats. The aerobic glycolysis was studied with the improved method of Warburg, using 14 Wistar rats weighing 150-225 gm. Half of these animals were injected subcutaneously with 2 mg of sodium-l-thyroxin daily for 3-4 days. The heads of 1 normal and 1 thyroxinized animal were then severed and brain slices prepared manually. These were studied with the above-mentioned method.

The $Q_{C_{O_2}}^{C_2}$ of the thyroxinized brain cortex slices was 3.9 as compared to 4.3 for control slices. The Q_{O_2} for the same brain slices was 10.6 for the thyroxinized as compared to 12 for the control slices. This small difference is at present not believed to be significant.

1442. SERUM PROTEIN BINDING OF RADIOACTIVE DIGITOXIN. J. L. Spratt,* G. T. Okita and E. M. K. Geiling. Dept. of Pharmacology and Argonne Cancer Research Hosp., Univ. of Chicago, Ill.

Investigations by various authors on the blood transport of digitoxin and its possible role in the cumulative action of cardiac glycosides have indicated a specific binding of the drug by serum albumin. A number of features of these reports (use of bioassay, use of nonspecific color reactions, high dosages and heterogeneity of proteins) require careful scrutiny in any attempt to interpret their data. Rothlin and Kallenberger's figure of 2.2 μg digitoxin bound per milligram human serum albumin (Arch. internat. pharmacodyn. 81, 1950) was based on the absence of an effect on the isolated frog heart. However, we cannot allow ourselves to attempt an in vivo application of this figure since it might imply that a normal man could bind 300 mg drug without significant pharmacological effect. Using non radioactive digitoxin at a dosage comparable to that employed in the above-mentioned studies and assayed by the Raymond colorimetric procedure, we have obtained results similar to those reported. However, when a near-therapeutic dose of biosynthetically labeled C^{14} digitoxin is used, one observes a binding of 0.01 μg/mg protein with a significant amount of degradation of the parent compound. When protein fractions are used, the radiotracer binding values do seem to be directly related to albumin content but also appear to be inversely related to cholesterol and phospholipid content of the protein samples.

1443. CLINICAL RESULTS WITH DIPHENYLTHIOCARBAZONE TREATMENT OF THALLIUM POISONING. W. B. Stavinoha, Joe B. Nash, Helen Davis and A. E. Hansen (intr. by G. A. Emerson). Depts. of Pharmacology, Toxicology and Pediatrics, Univ. of Texas Med. Branch, Galveston.

Oral administration of 10 mg/kg of diphenylthiocarbazone b.i.d., suspended in guar gum mucilage, results after 2 wk in abrupt clinical improvement in in fants poisoned with massive doses of thallium, particularly in regard to symptoms referable to neurotoxicity of Tl. In one severely poisoned infant, 40 mg/kg/day of dithizone in 2 divided doses caused exacerbation of symptoms, which later disappeared while the infant was maintained on 20 mg/kg/day of dithizone. These observations are in accord with

the antidotal and synergistic effects of dithizone in rats (Lund. Acta pharmacol. et toxicol. 12:260, 1956). No evidence of diabetogenic effects of dithizone was noted in urine or blood of any of the infants, which were uniformly given carbohydrate supplements before treatment. Effects of other sequestering agents and of -SH compounds, in animals, are described.

1444. THE RELATIVE THYMOLYTIC ACTIVITY OF CORTICOSTEROIDS IN THE IMMATURE RAT.
N. R. Stephenson (intr. by C. A. Morrell). Food and Drug Directorate, Dept. of Natl. Health and Welfare, Ottawa, Canada.

The influence of various alterations in molecular structure on the relative potency of 17-hydroxy-11-oxycorticosteroids was studied by means of the thymus involution method (Canad. J. Biochem. Physiol. 32:689, 1954). Intact immature male rats of the Wistar strain, 23-25 days old, were employed as the test animals. The corticoids were administered by subcutaneous injection 3 times daily for 2 days; the thymus gland of each rat was removed approximately 18 hr after the last injection of the corticoid and the weight in milligrams per 100 gm of body weight was recorded. The confidence interval of this type of assay, with 10 rats pwer dose group, was usually less than 0.1. The thymolytic activity of at least 4 compounds was determined in each assay. Thus the relative potency of 6 combinations of steroids were compared in a single test. By this assay procedure, 1-dehydro- 9 alpha-fluorohydrocortisone acetate is 16-17 times more active than hydrocortisone acetate, approximately twice as active as 9-alpha fluorohydrocorticone acetate, and about 5 times more active than prednisolone. Although the potency of 1-dehydro-11-oxycorticosteroids relative to 11-oxycorticoids is significantly greater when the compounds are injected in an aqueous medium than when they are given in corn oil, the introduction of a fluorine atom into the 9-alpha position of the molecule apparently abolishes this effect of the injection medium.

1445. METABOLISM OF URIDINE NUCLEOTIDES IN RELATION TO SITE OF ACTION OF PENICILLIN. Jack L. Strominger and Mildred Smith.*
Dept. of Pharmacology, Washington Univ., St. Louis, Mo.

Three uridine pyrophosphate N-acetylamino sugar compounds accumulate in penicillin-inhibited Staphylococcus aureus (Park. J. Biol. Chem., 194:877, 1952) in a manner which suggests a close relation to the site of action of penicillin (Strominger. J. Biol. Chem. Jan 1957). One of these compounds appears to be a precursor of the bacterial cell wall (Park and Strominger. Science. In press). Inhibition of bacterial growth and nucleotide accumulation may be the result of inhibition by penicillin at some point in the highly specialized reactions leading to cell wall synthesis. Crystal violet inhibits this sequence at a different point. From these studies it appears that uridine diphosphoacetylglucosamine (UDPAG) may be the parent of the nucleotides which accumulate with penicillin. An enzyme which catalyzes the synthesis of pyrophosphorolysis of UDPAG has been observed qualitatively in rat liver (Smith and Mills. Biochim. et biophys. acta 13:587, 1954): UTP+Ag-1-P UDPAG+PP. A quantitative assay for this enzyme has been developed. Its specific activity in extracts of S. aureus is 14.0, compared to values of 0.3 for calf liver acetone powder extract, 0.1 for hen oviduct and about 0.05 for calf liver. The liver enzyme has been purified 25-fold (specific activity, 7.4) from the acetone powder extract. Staphylococcal extract and purified liver enzyme cannot catalyze this reaction with uridine diphosphoacetylgalactosamine (UDPAGal) as substrate and therefore provide a basis of a method for separating UDPAGal from its naturally occurring mixture with UDPAG, the first available means of preparing UDPAGal in relatively pure form. (Aided by a grant from the Natl. Inst. of Arthritis and Metabolic Diseases, PHS.)

1446. A NEW HYPOTENSIVE STEROID. F. M. Sturtevant.
Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.

SC-6584 (17α-propyl-4,5 -dihydro-19-nortestosterone) was studied acutely and chronically in a total of 46 metacorticoid and metarenal hypertensive rats, blood pressures being determined without anesthesia or heating using a photoelectric tensometer. All experiments were conducted in a "blind" fashion with solvent-injected controls. SC-5684 significantly reversed the hypertension without causing any gross or histological toxic effects. In another experiment, SC-6584 was studied chronically in 7 normal and 6 DCA-implanted rats; 2 control groups received DCA alone and the solvent, respectively. There was a consistent tendency for SC-6584 to affect water and electrolyte metabolism in the direction opposite to the effect of DCA in both DCA and non-DCA treated animals. The SC-6584 + DCA rats had statistically significantly lower blood pressures than the DCA rats. No gross or histological toxic effects of SC-6584 were noted, although a total of 860 mg/kg were given in 44 days. This steroid has shown no appreciable hormonal activity or toxicity in any of the standard assays performed by our Division to date. The hypotensive action of SC-6584 is shared by the anabolic methyl homolog, but not by the unsubstituted compound, nor by the ethyl homolog even at doses 3 times that of SC-6584. A similar specificity of homologs has been observed previously in the 4,5-dehydro series, where the 17α-propyl and -ethyl (norethandrolone, Nilevar) derivatives were hypotensive, while nortestosterone and the 17α-methyl were inactive.

1447. EFFECTS OF GASTROINTESTINAL TRACT IRRADIATION ON Fe^{59} UPTAKE BY RATS.
Maurice F. Sullivan (intr. by Kenneth P. DuBois). Biology Operation, Hanford Labs., General Electric Co., Richland, Wash.

Tracer studies with radioiron have shown (Hennessy and Huff. Proc. Soc. Exper. Biol. & Med. 73: 436, 1950) that iron uptake by erythrocytes is a sensitive index of whole body X-radiation dosage. In the present study only the gastrointestinal tract, either in situ or after surgical exteriorization, was exposed to 250 kvp X-rays. The iron was administered intravenously as the citrated complex to rats. Serial measurement of the Fe^{59} content of the peripheral blood was made using direct counting techniques. A dose of 1500 r, which is approximately an LD_{30} when delivered to the intestine, caused only a slight decrease in iron uptake. Concomitant measurement of the amount of radioiron excreted in the feces showed that marked hemorrhaging into the intestine occurred between the 3rd and 10th day after exposure. It was at this time that ulceration of the intestinal mucosa was most in evidence. Administration by gavage of the beta emitter Y^{91}, which is not appreciably absorbed from the intestine, caused no greater decrease in iron uptake than that occasioned by the 1500 r of X-rays. The Y^{91} dosage (15 mc/kg) was of comparable toxicity to the X-ray dosage. It appears from these data that acute irradiation of the intestine has little

direct effect on erythropoiesis nor does it markedly inhibit or interfere with the absorption of metabolites essential to erythropoiesis.

1448. CARDIAC ARRHYTHMIAS FROM VERATRINE AND PROTOVERATRINE. Henry H. Swain and Duncan McCarthy, Jr. (intr. by M. H. Seevers). Univ. of Michigan, Ann Arbor.

Microgram quantities of veratrine or protoveratrine induce ventricular fibrillation in the dog heart-lung preparation. These substances differ, however, in their action on specific cardiac parameters. Protoveratrine increases cardiac automaticity and slows most types of intracardiac conduction but has relatively little effect upon the refractory period of the cardiac tissues. Doses of 20-40 μg increase spontaneous A-V nodal rate and also cause coupled ventricular beats. Auricle-to-auricle conduction, auricle-to-ventricle conduction and the Purkinje component of intraventricular conduction all are depressed by subfibrillatory doses of protoveratrine, while the myocardial component of intraventricular conduction is unchanged or even speeded at these doses. Atrial refractory period is prolonged by a small amount. Veratrine produces a striking prolongation of the refractory period of atrial muscle and the A-V conduction system. Rarely does veratrine cause premature ventricular beats. Veratrine effects upon conduction velocity are secondary to changes in refractory period of atrial and nodal tissues. After 500 μg of veratrine A-V conduction times on consecutive beats vary back and forth by as much as 20 msec and simultaneously the EKG shows alternation of QRS configuration. This alternation is dependent upon refractory period prolongation and is exaggerated at faster heart rates. After either protoveratrine or veratrine ventricular fibrillation is seen following doses of drug which depress the Purkinje conduction markedly while leaving the myocardial component of intraventricular conduction relatively unaffected. (Supported by Public Health Service Grant No. H-2466.)

1449. EFFECT OF 2,2-BIS-(PARA-ETHYLPHENYL)-1, 1-DICHLOROETHANE (PERTHANE) ON ADRENOCORTICAL FUNCTION IN HUMANS. I. Taliaferro and L. Leone (intr. by H. B. Haag). Depts. of Pharmacology and Medicine, Med. College of Virginia, Richmond.

Perthane is an insecticide related to DDD, which has been shown to produce adrenocortical atrophy in the dog. Because adrenalectomy favorably influences certain hormone-dependent malignancies, Perthane has been given to 13 patients with metastatic carcinoma of the breast or prostate to test its effect on the human adrenal. The compound was given orally in corn oil over periods ranging from 2 days to 12 wk. Plasma 17-hydroxycorticosteroid values were determined in 11 patients as an index of adrenocortical function. When the initial values were within the nomral range, 50 mg/kg/day of Perthane caused no change (2 patients). When initial values were elevated due to previous stilbestrol therapy, a fall in these values was seen following Perthane (2 patients). Response of plasma 17-hydroxycorticosteroid levels to standard ACTH tests was diminished during Perthane administration (2 patients). When a 9-day course of Perthane (200 mg/kg/day) was superimposed during continuous ACTH stimulation (22 days) a prompt and marked fall in 17-hydroxycorticosteroid levels was found (1 patient). Excretory products of Perthane interfered with urinary steroid determinations. One serious but reversible toxic reaction, thrombocytopenia and leucopenia, has been encountered. The data are interpreted as indicating that Perthane inhibits adrenal responsiveness to ACTH in humans. (Aided by grants from Natl. Heart Inst. (H-2376), and Natl. Cancer Inst., PHS (CT-496).)

1450. A NEW METHOD FOR TESTING FUNGICIDAL ACTION IN VITRO. Giok Hoa Tan,* Thomas N. Burbridge* and Hamilton H. Anderson. Dept. of Pharmacology, Univ. of California School of Medicine, San Francisco.

Accurately measured Sabouraud agar slants were inoculated with 3-wk stock cultures of T. mentogrophytes or 4-wk cultures of H. capsulatum and incubated at 30°C. After growth was sufficient, those tubes containing the most uniform colonies were used for testing. Appropriate concentrations of the drug to be tested were prepared in a nonfungicidal solvent. A selected amount of each concentration was used to "flood" the colonies in each of 4 tubes. At time intervals varying from 5 min to 72 hr, a small amount of the mycelia from each of the 4 tubes was subcultured on 2 tubes so that, at a given time interval for each concentration, 8 subcultures were made to determine the fungicidal activity. Since there was danger of transferring the fungicidal agent during subculture, this was minimized by spreading the mycelia over the agar slant surface. Controls were processed simultaneously using the solvent without fungicide. The advantages are: 1) the required exposure time is determined; 2) problems of diffusion in artificial media are eliminated; 3) only fungicidal activity is determined. Examples of the usefulness of the method are: 1) a salicylic acid derivative was found to be potent to T. mentogrophytes in low concentrations only after 48 hr exposure; 2) a salicylanidid derivative was fungistatic for H. capsulatum at 1:300,000 (using the toxic agar method) but not fungicidal at 1:10,000 (our method).

1451. HEPATIC REGULATION OF CARDIAC GLYCOSIDE ACTION. Tsuneyoshi Tanabe and Mamoru Tanaka (intr. by M. H. Seevers). Sapporo Med. College, Sapporo, Japan and Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Isolated rat and guinea pig liver was perfused with Locke's solution containing a given concentration of cardiac glycosides. Perfusion fluid was then collected from the hepatic vein and assayed for glycoside content using embryonic chick heart. The uptake of glycoside by liver tissue was enhanced with repeated perfusions and occurred as follows: digitoxin > lanatoside c > g-strophanthin > convallatoxin. This corresponds to lethal dose order of these substances. In animals poisoned with CCl_4 or injected with india ink, liver uptake was markedly depressed. The lethal dose of digitoxin in cats and guinea pigs was increased when extracts of liver homogenates were injected prior to digitoxin administration. In addition, a pronounced bradycardia was observed in digitoxin treated cats previously injected with liver extracts. Atropine abolished these effects. Kidney extracts did not alter the response to digitoxin. The results of this study indicate that some substance normally present in liver tissue reduces the lethal action of cardiac glycosides and promotes the development of bradycardia in response to digitoxin administration. It is suggested that the liver plays an important role not only in the detoxication of cardiac glycosides but also in regulating the cardiac response to administration of these drugs.

1452. CARDIOTONIC STRUCTURE-ACTIVITY RELATIONSHIP OF THE STEROID NUCLEUS. Ralph

D. Tanz* and Richard W. Whitehead. Dept. of Pharmacology, Univ. of Colorado School of Medicine, Denver.

Previous work from this laboratory has shown the glycoside-like activity of 9-alpha fluorohydrocortisone (9-α FF) on the papillary muscle and isolated whole heart preparations. This cardiotonic action has been further correlated with the intramuscular movement of Na^{22} and K^{42} as well as histology. In addition to 9-α FF we have screened the synthetic steroid Viadril, cortisone, estrone and to a lesser extent DOCA and Nilevar in the same preparations. Under our experimental conditions a steroidal substance shows cardiotonic activity when it is able to produce a positive inotropic action on the fatigued or failing tissue in a concentration of about 1.0 μg/m; or perfusate. Conversely, a negative inotropic action occurs from concentrations above 10 μg/ml. In addition, those agents (in proper concentrations) producing a positive effect cause an increased intramuscular uptake of Na^{22} and a loss of K^{42}, whereas a reversal in this shift occurs when a negative inotropic action results. 9-α FF, cortisone, DOCA and Nilevar apparently possess cardiotonic activity, while Viadril and estrone do not. This information, plus that of Emele's (Am. J. Physiol. 185:103, 1956), results in a steroid nucleus (cyclopentanoperhydrophenanthrene) having a =O at C-3, a methyl group at C-18 and C-19, and a 2-carbon side chain at C-17. This structure possesses those radicals common to all cardiotonically active steroids tested.

1453. EFFECT OF HYPOPHYSECTOMY ON ADRENAL ASCORBIC ACID RESPONSE TO ASPIRIN. Joan K. Taylor* and E. Leong Way. Dept. of Pharmacology, Schools of Pharmacy and Medicine, Univ. of California, San Francisco.

It has been recently reported that the hypothalamus is an intermediary for pituitary-adrenal activation by aspirin. Lesions in the anterior and middle regions of the median eminence blocked the adrenal ascorbic acid depleting effects of aspirin in the rat (George and Way. J. Pharm. In press). These results are compatible with the earlier findings that the integrity of the adenohypophysis is essential for the response to aspirin. In these studies comparisons were made on a series of lesioned animals given either aspirin or saline. However, it has been recently reported (Coste et al. 1953; Widemann and Petersen, 1955) that hypophysectomy does not prevent the adrenal ascorbic acid reducing effects of aspirin. Control adrenal ascorbic acid levels were obtained by removing one adrenal from the animals under anesthesia. The ascorbic acid in the remaining adrenal was reported to be decreased following aspirin administration. Since these findings are not consistent with the earlier literature, it was felt that the experimental conditions may have enhanced sensitivity to aspirin. However, in experiments initiated to duplicate the procedure of the above workers we were unable to confirm their findings. (Supported, in part, by a grant from the Inst. of Analgesic and Sedative Drugs.)

1454. PLASMA AND URINE 17-HYDROXYCORTICOSTEROID AND URINE 17-KETOSTEROID LEVELS IN MAN DURING ACCLIMATIZATION TO HIGH ALTITUDE. P. S. Timiras, N. Pace* and C. A. Hwang.* Dept. of Physiology, Univ. of California, Berkeley and the White Mountain Research Station, Big Pine, Calif.

Studies were made on 6 healthy adult males during the summer of 1955. The subjects went from their residence in Berkeley (250 ft.) to the Barcroft Laboratory (12,470 ft.) of the White Mountain Research Station for 3 separate sojourns of 5-8 days each with intervening periods at sea level. All urine was collected throughout successive 12-hr periods for 1-3 days in the week preceding each exposure and every day at altitude. Venous blood specimens were obtained before breakfast. After exposure to altitude, urinary excretion of 17-hydroxycorticosteroids and of 17-ketosteroids was increased by as much as 300% and 50%, respectively, over sea level values. Similarly, plasma levels of 17-hydroxycorticosteroids were higher at altitude than at sea level with a maximum increase of 115%. On the other hand, the urine and plasma levels of adrenocortical hormones and their metabolites appeared to be related to the duration of the exposure and the degree of acclimatization. They rose during the first 3 days of each sojourn at altitude and then returned toward the sea level values. In addition, the magnitude of these changes decreased in successive sojourns. The observations suggest that exposure to altitude stimulates in man adrenocortical activity but that the increase in the levels of the adrenocortical hormones is only temporary. (Supported by Office of Naval Research contract.)

1455. OCTOIODOFLUORESCEIN AS AN AGENT FOR LOCALIZING BRAIN TUMORS IN MICE. Edward C. Tocus* and George T. Okita. Argonne Cancer Research Hosp. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.

The evaluation of a substance for brain tumor localization is based on the differential uptake between normal and tumor tissue. Octoiodofluorescein (OIF) labeled with I^{131} was found to localize in transplantable Swiss mouse brain tumors (ependymoblastoma) with a ratio of from 17 to 32 times that for an equal weight of normal brain tissue 1 hr after injection. The amount per gram of brain tumor was from 1.5 to 9.5% of the injected dose, for normal brain tissue from 0.086 to 0.39% with a mean value of 0.14% of the injected dose after 1 hr. The ratio appears greater after 24 hr due to the decrease to zero concentration in the normal brain tissue with slight decrease in the tumor. OIF was found to be opaque to X-rays in the range of concentrations found in experimental brain tumors; thus it is believed to have value as an agent which can visualize brain tumors when given intravenously at nontoxic doses. The i.v. LD_{50} dose for adult C3H female mice was 510 mg/kg. After 24 hr, 52% of the injected OIF was found in the feces and 0.8% in the urine. Distribution studies showed after 4 hr, 10.9% in the liver, 0.36% in the spleen, 0.96% in the kidneys, 0.0% in the brain and 2% of the injected dose in 1 ml of blood. The liver reached a maximum concentration of 39.7% of the dose 20 min after injection. Single sublethal doses had no effect on the life span or reproduction of CF-1 female and male mice.

1456. ROLE OF ALERTING IN APPARENT ACTIONS OF DRUGS ON CORTICAL EVOKED POTENTIALS IN RABBITS. J. E. P. Toman, G. M. Everett and A. H. Smith, Jr.* Dept. of Physiology and Pharamcology, Chicago Med. School and Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.

Unanesthetized uncurarized rabbits with implanted needle electrodes were used for study of evoked EEG response recorded from points both adjacent and contraplateral to the site of stimulus on sensorimotor cortex. Typical adjacent response to adequate shocks includes brief "A" type spikes, a 10-msec negative component of presumed "dendritic" origin, a 200-msec negative "slow" wave envelope and a train of biphasic

10/sec waves having a form characteristic of sleep "spindles." Of these, the "spike" components are very insensitive both to various drugs and to "alerting" stimuli (airblast, moving visual object, sound, pain). Both the "dendritic" and "spindle" responses are enhanced by sedative barbiturates and reduced by alerting stimuli. The "slow" component is enhanced by various excitants but reduced, at least in extent of cortical response zone, by alerting; some drugs may produce apparent changes in local response which are secondary to alerting. Thus both d-tubocurarine and succinyl choline paralysis may be associated with a maintained alert EEG and with suppression of "dendritic" and "spindle" evoked responses. However, these "blocked" responses may then be restored by pentobarbital sufficient to permit light sedation. Under these conditions the typical alerting changes may again be evoked as in the normal rabbit, indicating normal cortical function in the curarized rabbit.

1457. NICOTINE EFFECTS ON NORMAL AND ATHEROSCLEROTIC RABBIT HEARTS. Janet Travell, Dorothy Karp and Seymour H. Rinzler. Dept. of Pharmacology, Cornell Univ. Med. College, New York City.

Electrocardiographic effects of nicotine 0.05 mg/kg (injected intravenously as nicotine bitartrate during pentobarbital anesthesia) were studied in 12 normal rabbits and 16 cholesterol-fed rabbits with coronary artery disease. That the latter had developed coronary atherosclerosis was evidenced by a positive ergonovine test (Rinzler et al. Am. J. Physiol. 184:605, 1956) and subsequent pathologic examination. Nicotine caused sagging depression of the S-T segment, similar to that induced by ergonovine, in about 10% of rabbits with coronary artery disease. In control animals nicotine did not alter the S-T segment. Occasionally, lowering of the T-wave was seen after nicotine, but spontaneous T-wave changes occur so often in rabbits that they cannot be regarded as indicative of drug action. No consistent effects of nicotine on heart rate were noted. Following electrocardiographic studies, several normal and atherosclerotic animals were selected for perfusion of the isolated heart by a modified Langendorff technique. Initially, atherosclerotic hearts had a higher coronary flow, slower heart rate and lower amplitude of contraction than normal hearts. Nicotine in graded doses of 0.01-0.1 mg was injected directly into the perfusion system. As with ergonovine (Karp et al. XXth Internat. Physiol. Cong., Brussels, 1956, Abstr. of Communications, p. 482), normal and atherosclerotic hearts showed differences in their response to nicotine, especially with respect to coronary flow.

1458. EFFECT OF CORTICOSTEROIDS ON CATALASE ACTIVITY IN LIVER HOMOGENATES. Robert C. Troop (intr. by Robert A. Woodbury). Dept. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.

Previous experiments in this and other laboratories have shown that cortisone and hydrocortisone produce a depression of liver catalase activity when administered to the intact rat. In this investigation depression of catalase activity by these steroids has been observed in an in vitro system. Rat liver was homogenized in Ringer-phosphate buffer solution (pH 7.4) to give a 1:500 homogenate. The steroid to be tested was incubated with 10 cc of this homogenate for 2 hr at 37°C under aerobic conditions. Control samples of the homogenate were incubated without added steroid. Cortisone produced a marked inhibition of catalase activity. The magnitude of this depression ranged from 22% at a cortisone concentration of 2.5×10^{-4} M to 80% at 5.6×10^{-3} M cortisone. Half inhibition occurred at a cortisone concentration of approximately 1.2×10^{-3} M. Incubation of the homogenate with hydrocortisone also caused depression of catalase activity but to a lesser degree than with cortisone. Half inhibition was obtained when the hydrocortisone concentration was approximately 2.9×10^{-3} M. This finding is of special interest since our in vivo studies also showed cortisone to be more effective than hydrocortisone in depressing catalase activity. Cortisone inhibition of catalase does not occur if incubation is carried out at 0°C. Cortisosterone at 1.7×10^{-3} M concentration produced no apparent effect. (Supported in part by Grant C-3191 from the Natl. Insts. of Health, PHS.)

1459. LOCAL ANESTHETIC STUDIES ON ISOLATED FROG SCIATIC NERVES. A. P. Truant. Astra Biological Labs., Worcester, Mass.

The blocking potency of certain local anesthetics was determined by the action potential technique utilizing the isolated sciatic-peroneal preparation from the frog (Rana pipiens). The nerve was maximally stimulated at a frequency of 10 and 30/sec. Before each test the normalcy of the nerve was determined by checking the excitability threshold, latency, degree of Wedensky and spike amplitude. Millimolar concentrations of the drugs were made up in Ringer's solution adjusted to pH 7.2, and applied to the nonbranched central portion (1.5 cm) of the nerve. Chamber temperature was maintained at 22°-23°C. The potency of an agent was established by determining the millimolar concentration necessary to linearly decrease the height of monophasic A spike by 50-60% in 8-10 min. The recovery process was followed by recording the spike height at 1-2 min intervals, during a constant Ringer wash. Nerves could be used for 6-8 experiments, except those with tetracaine and dibucaine. The following millimolar blocking concentrations were established: lidocaine (2.5), procaine (8), procaine + propoxycaine in a ratio of 5:1 (2.8), tetracaine (0.6), dibucaine (0.2). With lidocaine and procaine, the recovery process began within 1-2 min and was completed within 20-30 min. However, with procaine + propoxycaine, tetracaine and dibucaine, the recovery was very slow and variable (40-100 min). Binding experiments on brain, nerve and heart homogenates support the hypothesis that the delayed recovery seen with propoxycaine, tetracaine and dibucaine is due to their relatively high binding capacity. The in vivo characteristics of these local anesthetics will be discussed.

1460. ANTI-UTEROTROPHIC EFFECT OF LARGE ORAL DOSES OF ESTROGENS IN THE IMMATURE MOUSE. Ernest J. Umberger,* George H. Gass* and Jack M. Curtis. Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

The uterine weight response to a wide range of doses of estrogens incorporated in the diet of immature female mice for a period of 7 days was studied. At extremely low dosage levels, linearity is achieved when the dose is plotted vs. the log of the uterine weight. At intermediate dosage levels, the relationship becomes linear when the log of the dose is plotted vs. the uterine weight. The dose-response curve then plateaus. If the dosage is increased beyond the plateau level, there is a diminished response of the uterus to estrogenic stimulation. The dosage level at which plateauing occurs and at which the anti-uterotrophic effect begins parallels the relative potency

of the estrogen. The assay method in the low dose range is 10-20 times as sensitive for stilbestrol as it is for the natural estrogens, estradiol and estrone.

1461. EFFECT OF X-IRRADIATION AND OTHER HEMATOPOIETIC DEPRESSANTS ON AMINO ACID INCORPORATION AND CARBOHYDRATE METABOLISM OF RABBIT BONE MARROW. Edwin M. Uyeki* and Paul R. Salerno. Atomic Energy Med. Research Project, Western Reserve Univ., Cleveland, Ohio.

Bone marrow cells from male albino rabbits were isolated from supporting tissue by means of a syringe fitted with a wire mesh screen and were incubated in a Krebs-Ringer solution containing glucose, C^{14}-labeled leucine and other amino acids. The incorporation of labeled leucine into protein was studied simultaneously with manometric determinations of anaerobic and aerobic glycolysis. The values were expressed on the basis of comparable numbers of cells. The incorporation of labeled leucine into protein in bone marrow cells was studied under anaerobic and aerobic conditions. Aerobically, 0.01 M KCN abolished the incorporation of labeled leucine; anaerobically, 0.01 M KF produced complete inhibition. 10^{-3} M and 10^{-4} M dinitrophenol decreased the oxygen consumption of bone marrow cells with a concomitant inhibition of 80-90% inhibition in the amino acid incorporation. $2.x10^{-5}$ M dinitrophenol resulted in a 60% increase in oxygen consumption and a 50% decrease from the control value for the amino acid incorporation into protein. 10^{-3} M and 10^{-4} M quinacrine hydrochloride inhibited the incorporation of labeled leucine into protein by 93 and 40%, respectively, whereas 10^{-5} M quinacrine exhibited no significant inhibitory activity. Male albino rabbits were exposed to 400 r and 800 r of X-irradiation and killed 1, 2 and 3 days later. It was found that the incorporation of labeled leucrine into protein, based on comparable numbers of cells, was significantly reduced. The possible relationship between these results and the altered cytology of the bone marrow is also being considered with parallel morphologic studies.

1462. EFFECT OF ENDOTOXINS ON VASCULAR REACTIVITY. R. Vargas and L. Beck (intr. by M. H. Seevers). Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Endotoxins of Salmonella typhosa and E. coli have been studied for their effect upon vascular reactivity in the intact animal and upon the isolated arterial strip. Little or no difference was seen in the response to epinephrine, norepinephrine or isopropylarterenol during the hypotensive response to the endotoxin or after the blood pressure has returned to normal. No effect was seen on the isolated arterial strip. The peripheral resistance in the perfused hind leg of the dog rises during the hypotensive response produced by the endotoxin and returns toward normal as the blood pressure recovers, indicating an increase in sympathetic activity during hypotension. The effects of the endotoxin upon other vascular beds are at present under investigation. Additional evidence for the presence of sympathetic activity during the hypotensive response to endotoxin is seen by a further decrease in blood pressure upon the administration of a ganglionic blocking agent. Large doses of endotoxin failed to depress the isolated perfused rabbit heart. (Supported by the Natl. Heart Inst., PHS, Grant No. H2578.)

1463. CHANGES FOLLOWING THE ACUTE ADMINISTRATION OF METHOCARBAMOL (NEURAXIN) (MOTION PICTURE). Francis A. Vazuka, Norma Hajek-Nichols and Jack Freund (intr. by P. S. Larson). McGuire VA Hosp. and Med. College of Virginia, Richmond.

Individuals with neuromuscular disorders were observed photographically prior to, during and following the intravenous administration of Methocarbamol. The changes in functional capacity as induced by drug administration were recorded. The subjects showed improvement in activity following reflex stimulation, ambulation capabilities and self-care.

1464. EFFECT OF SOME SUBSTANCES OF METABOLIC INTEREST ON ISOLATED AURICLES OF THE RAT. V. M. Venturi and Hollis G. Schoepke (intr. by F. E. Shideman). Dept. of Pharmacology and Toxicology, Univ. of Wisconsin, Madison.

The isotonic contractile amplitude of the isolated auricles of the rat is reduced by various members of the Krebs cycle (citrate, α-ketoglutarate, succinate, l-malate). A qualitatively similar effect is produced by malonate, an inhibitor of this cycle, as well as by dl-lactate, pyruvate and acetate. These compounds were tested in concentrations ranging from 1.0 to 10.0 μM/ml and inhibitory activity was found to be greatest in the case of citrate and α-ketoglutarate and least in the case of lactate, pyruvate and acetate. Results obtained following the addition of Ca^{++} to the medium after the effects of any one of the above chemical substances had become established, suggest that they effects of the latter may be related to their ability to chelate calcium. Experiments are being conducted to establish this point and to provide comparable data on isolated ventricular strips.

1465. INHIBITION OF RENAL TUBULAR EXCRETION OF N^1-METHYLNICOTINAMIDE (NMN) BY OTHER ORGANIC BASES IN THE CHICKEN. R. L. Volle,* R. E. Green and Lawrence Peters. Dept. of Pharmacology, Kansas Univ. Med. School, Kansas City.

The simultaneous renal tubular excretion of NMN and p-aminohippurate (PAH) by hens was studied using Sperber's procedure (Ann. Royal Agric. Coll. Sweden, 15:317, 1948). (See abstr. by Green and Peters). Tetraethylammonium (TEA) caused a 60% inhibition of the renal tubular excretion of NMN when the molar ratio of TEA:NMN infused was 7.5:1. The readily reversible inhibition was less marked but highly significant when the infusion ratio was 5:1 and was absent when the ratio was 2.5:1. Priscoline (tolazoline) produced a 60% inhibition of NMN transport when the molar ratio of Priscoline: NMN infused was 2.5:1, and an 87% inhibition when the ratio was 7.5:1. When Darstine (mepiperphenidol) and NMN were infused together in a molar ratio of 2.5:1, NMN transport was completely inhibited. It was also completely inhibited when cyamine dye #863 (1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride) and NMN were infused in a molar ratio of 0.18:1. The renal tubular excretion of PAH was not affected by the organic bases under the experimental conditions employed. The relative affinities of the various bases studied for the transport system involved will be discussed on the basis of the data presented. (Supported by Grant No. H-2377 from the Natl. Heart Inst., PHS.)

1466. A SIMPLE TEST TUBE METHOD FOR THE PRODUCTION OF ALL-HUMAN FIBRINOLYTIC MATERIAL. Kurt N. von Kaulla and Charles Taylor (intr. by William B. Draper). Dept. of Medicine, Univ. of Colorado School of Medicine and

Belle Bonfils Mem. Blood Bank, Denver.

Treatment of thromboembolic disorders by activation by pyrogens of native human fibrinolytic system is a possibility. A convenient test tube model is desirable for studies on the effect of various drugs on this system. We found that substitution of dialyzed urine for distilled water in precipitation of human plasma euglobulins reduces their spontaneous lysis time from hours to a few minutes. Based on this observation the following method was developed: 1 part plasma (yielding more potent preparations) or serum (both from outdated bank blood) was added to 20 parts of fresh voided clear unfiltered human urine, spec. grav. > 1.015; the mixture was dialyzed in a rocking device against running tap water at 4-7°C for 2 hr, then saturated with CO_2. A clot composed of 1 part CO_2-precipitated redissolved activated euglobulins and 1 part of original plasma lysed in 15-60 min, a 1:4 mixture in 24 hr. One part plasma in 100 parts urine yielded preparations which dissolved clots of 10 parts plasma in a few hours, some activation was demonstrable in a !;4 mixture. Increasing dialyzing temperature reduced potency; replacement of plasma by plasminogen had no clear advantage. Fibrin plates revealed presence of potent plasmin in urine-activated euglobulins. In testing for activator content of urine with activated euglobulins, human standard plasma clots reflect this activity better than bovine fibrin clots. (Supported by grants from the American Heart Assoc. and the Belle Bonfils Mem. Blood Bank, Denver.)

1467. SEX DIFFERENCE IN AORTIC RUPTURE INDUCED BY LATHYRUS ODORATUS IN IMMATURE RATS. Isabel Wajda,* David Lehr and Marilyn Krukowski.* Dept. of Physiology and Pharmacology, New York Med. College, Flower and Fifth Ave. Hosps., New York City.

In search for the mechanism of medionecrosis and aneurysm formation in the rat aorta produced by standard renal injury (Lehr et al. Bull. New York M. Coll. 15:130, 1952), we reinvestigated the aortic lesion occurring after ingestion of sweet pea seeds in immature rats (Bachhuber and Lalich. Science 120:712, 1954). Groups of male and female weaning rats received a synthetic diet containing 50% ground Lathyrus odoratus seeds. Preliminary results based on the first completed experiment comprising 40 rats were as follows: There was no significant difference between the sexes regarding food intake and retardation of weight gain and all animals developed the typical severe skeletal deformities. However, while most of the male rats were dead from aortic rupture by the 5th wk, less than 50% of female animals had succumbed by that time, the remainder surviving substantially longer. In addition, the overall incidence of aortic rupture was considerably lower in female animals. In line with this sex difference, male rats revealed statistically significant increases in the moist weight of testes, adrenals, kidneys and hearts as compared to the organ weights of heavier control litter mates raised on Purina chow, whereas no comparable increase in organ weights was found in the female group. It was assumed, therefore, that a true sex hormonal difference may be involved, despite the immaturity of these animals. It is of particular interest, in this connection, that androgens were shown to enhance considerably the incidence and severity of aortic medionecrosis produced by our method. (Lehr and Martin. Proc. Soc. Exper. Biol. & Med., In press.) (Supported by research grant No. H-890 (C5) from the Natl. Heart Inst., PHS.)

1468. MEGIMIDE AS A DORIDEN ANTAGONIST. Donald T. Walz,* Frederick F. Cowan, Jr.* and Theodore Koppanyi. Pharmacology Dept., Georgetown Univ. Med. and Dental Schools, Washington, D. C.

In mice, the i.p. LD_{50} of alpha-ethyl-alpha-phenylglutarimide is 182 mg/kg, and that of beta-beta-methyl-ethyl-glutarimide is 29 mg/kg. One LD_{50} of Megimide does not antagonize 1 LD_{50} of Doriden. Two LD_{50}'s of Doriden are not antagonized by 4 LD_{50}'s of Megimide. The cause of death is CNS depression. One LD_{50} of Doriden, followed by 6 LD_{50}'s of Megimide, resulted in fatal convulsions, indicating that now the Megimide convulsant action supervened. Positive results were obtained when the ratio of Doriden to Megimide was 1:4 (only if 1 = 1 LD_{50} of Doriden). It was found then that the average awakening time is about 13 min, with the animals staying awake and not lapsing back into coma. However, when 2 LD_{50}'s of Doriden were administered, 8 LD_{50}'s of Megimide failed to modify Doriden toxicity—still a ratio of 1:4. It is therefore evident that Megimide is not a competitive antagonist to Doriden. Nevertheless, the striking response following administration of 1 LD_{50} of Doriden and 4 LD_{50}'s of Megimide indicate the real and undeniable physiological antagonism between the 2 drugs. Both Doriden and Megimide are long-acting drugs. One LD_{50} of Doriden depresses the animals for about 24 hr, and handling of the animals produces seizures several hours following the simultaneous administration of Megimide and central depressants. Megimide was also successfully used in the treatment of human Doriden intoxication.

1469. NUTRIENT AND ENERGY METABOLISM IN PATIENTS WITH AND WITHOUT CANCER DURING HYPERALIMENTATION WITH FAT ADMINISTERED INTRAVENOUSLY. Donald M. Watkin and Jesse L. Steinfeld (intr. by Morris Belkin). Nutrition and Metabolism Service, Natl. Cancer Inst., Bethesda, Md.

Four patients with cancer and 4 with chronic illnesses other than cancer were infused daily for 36 days with 600 ml of a 50% cottonseed oil emulsion while maintained on a constant oral intake. Balances of nitrogen, potassium, phosphorus, calcium, sodium and chloride were measured before, during and after the period of fat administration. Calorie balances were estimated by the insensible water loss method of Newburgh. Patients with and without cancer responded to hyperalimentation with positive balances of nitrogen, potassium, phosphorus and sodium. Both groups gained weight. However, patients with cancer, in negative caloric balance during control periods, increased their caloric expenditure during hyperalimentation sufficient to remain in negative caloric balance. In contrast, patients without cancer, in caloric equilibrium during the control period, responded to hyperalimentation with positive caloric balances. Patients with cancer lost weight rapidly when hyperalimentation was withdrawn while patients without cancer tended to maintain the weight gained. Fecal fat excretion did not rise significantly in any patient. The metabolic mixtures burned by patients with cancer contained a higher proportion of fat, estimated by Lavietes' formula, than those of noncancer patients. Studies with I^{131}-labeled human serum albumin revealed an increase in total exchangeable albumin and its turnover rate in both cancer and noncancer patients during hyperalimentation. BSP retention and a moderate anemia which developed during

hyperalimentation disappeared when the fat infusions were discontinued.

1470. COMPARATIVE STUDY OF PERIPHERAL EFFECTS OF FLUOTHANE, CYCLOPROPANE, ETHER AND CHLOROFORM ON MUSCULAR CONTRACTION IN RABBITS. D. C. Watland,* J. P. Long and C. B. Pittinger. Dept. of Surgery, Div. of Anesthesiology and Dept. of Pharmacology, State Univ. of Iowa College of Medicine, Iowa City.

Peripheral inhibition of muscular contraction caused by ether is well established (Gross and Cullen. J. Pharmacol. & Exper. Therap. 78:358, 1943; Poulsen and Secher. Acta pharmacol. 5:196, 1949; Secher. Acta pharmacol. 6:371, 1950). Neuromuscular effects of other anesthetic agents are less clarly defined. The peripheral effect of Fluothane (2-bromo-2-chloro-1, 1,1-trifluoro-ethane) on muscular contraction has not been reported and is the subject of this study. Rabbits were prepared during anesthesia for stimulation of the severed sciatic nerve and recordings of isometric contractions of gastrocnemius muscle and femoral arterial blood pressure. A Grass stimulator provided supra-maximal, single shock impulses every 10 sec. Comparative effects of Fluothane, cyclopropane, ether and chloroform were studied. Ether consistently depressed gastroncnemius contractions and the depressant effect persisted after awakening. Fluothane, cyclopropane and chloroform tended to enhance muscular contractions and their effects were less persistent. Fluothane and chloroform consistently lowered the blood pressure of the rabbits. Effects of ether and cyclopropane on blood pressure were variable.

1471. PERIPHERAL BLOOD EPINEPHRINE LEVELS FOLLOWING INTRAVENOUS ADMINISTRATION OF THE MATERIAL. D. T. Watts and T. R. Poole.* Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgantown.

Peripheral blood epinephrine levels and arteriovenous differences have been determined during the constant infusion and after single inv. injections of increasing quantities of epinephrine. Epinephrine determinations were made by the bioassay method of Gaddum and Lembeck (Brit. J. Pharmacol. 4:401, 1949) which employs the automatically stimulated rat uterus and is sensitive to about one μg/l. epinephrine. The following values, showing infusion rate in μg/kg/min and venous blood epinephrine in μg/l. respectively, are typical results: 1.25, 5.7; 2.5, 11.0; 5, 33.9; 10, 74.8. At a given rate of infusion blood epinephrine levels remained constant for infusions lasting up to 3 hr. This indicates epinephrine is either destroyed or removed from the circulation as rapidly as it was administered. At low rates of infusion, i.e., 1 μg/kg/min the arteriovenous difference in epinephrine was small, whereas at 5 μg/kg/min the levels were 27.2 and 74.5 μg/l. in venous and arterial blood respectively. After the administration of a single i.v. dose of epinephrine in the range of 1-100 μg/kg venous plasma epinephrine closely parallels blood pressure changes and has returned to pre-injection levels at the end of 2-3 min. (Aided by grants from the Natl. Heart Inst., PHS and the Life Insurance Med. Research Fund.)

1472. ESTIMATION OF HEROIN AND MORPHINE IN BIOLOGIC FLUIDS. E. Leong Way. Pharmakologisches Institut, Universitat Bern, Bern, Switzerland.

In order to study the metabolism of heroin in the rat and in man, a method was developed for estimating heroin at microgram quantities in biologic fluids. Since heroin is known to be metabolized in vivo to morphine, the procedure was developed for application in the presence of a mixture of the two compounds. The procedure depends on the selective extraction of heroin from biologic media with 10% normal butanol in chloroform at pH 8.5, hydrolyzing the compound to a phenolic substance, and estimating it photometrically as morphine with the Folin-Ciocalteu reagent. Satisfactory receovery of heroin added to tissue is obtained with relatively little emulsion formation and low tissue blanks. Morphine is also extracted from tissue by the solvent under these conditions. However, the morphine can be separated from the heroin by extracting the solvent with a phosphate pH 5.8 buffer which removes only the morphine. The heroin is subsequently removed from the solvent with N/2 sulfuric acid. Sensitivity of the method is about 2 μg. (Supported, in part, by a grant from the PHS.)

1473. ENZYMATIC SYNTHESIS OF NUCLEOTIDES OF PURINE ANALOGS. James L. Way* and R. E. Parks, Jr. Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison.

Nucleotides of various purine analogs have been synthesized from their respective bases in the presence of enzyme preparations from liver and yeast by the method of Kornberg, Liebermann and Sims (J. Biol. Chem. 215:417, 1955). The compounds, 8-azaguanine and 8-azaxanthine, have been converted to their respective 5'-nucleotides via the intermediate 5-phosphoribosylpyrophosphate (PRPP) which was prepared by the method of Remy, Remy and Buchanan (J. Biol. Chem. 217:885, 1955). Incubation of PRPP and the purine analogs with extracts of hog or beef liver acetone powders resulted in the formation of 5'-nucleotides in good yield. The unreacted bases were separated from the nucleotides by the use of Dowex-50-H^+ ion exchange columns. The nucleotides were then adsorbed on Darco S-51 charcoal columns and eluted with ammoniacal ethanol. These nucleotides had ratios of base:ribose:phosphate of approximately 1:1:1, and upon reaction with snake venom 5'-nucleotidase yielded inorganic phosphate and the expected nucleoside. The purine analogs, 6-Mercaptopurine, 2,6-diaminopurine, 8-azaadenine, and the guanine, adenine and hypoxanthine analogs of the pyrazolo(3,4-d) pyrimidine series were similarly incubated in the presence of liver and yeast preparations and their nucleotides were identified by paper chromatography. Attempts to synthesize polyphosphate nucleotides of the above analogs have been initiated.

1474. DERMAL TOXICITY OF ETHANOLAMINES IN TOPICAL PREPARATIONS. Francis X. Wazeter,* Elsie A. Kelley,* John H. Draize and Robert E. Zwickey. Div. of Pharmacology, Food and Drug Admin., Washington, D. C.

Dermal toxicity of 2-aminoethanol,2,2'-iminodiethanol, and 2,2',2''-nitrilotriethanol was studied in the rabbit. These compounds were tested individually as the free amines, in aqueous solution and in hydrophilic and white ointment bases on intact and abraded skin. Daily doses in the 90-day studies varied from 10 to 80 mg of amine/kg of body weight for the 2% aqueous media, and from 250 to 500 mg of amine/kg of body weight for the 5% ointments. In addition, the compounds were administered for 3 wk by the oral route at daily doses ranging from 50 to 500 mg/kg of body weight. Body weights, food intake and gross local effects were recorded daily. Urines were examined periodically during the course of all experiments

for the presence of protein. Complete blood counts were determined 3 times for rabbits on the 90-day studies. Tissues of rabbits on all experiments were examined histologically and included the kidney, liver, heart, skin, bone marrow, spleen and thyroid. Gross observation revealed no significant toxic or other adverse side effects. Similarly, no microscopic pathological changes have been seen in tissues examined.

1475. CARDIOVASCULAR AND BRONCHIOLAR PROPERTIES OF NARCOTINE HYDROCHLORIDE. Lawrence C. Weaver, William M. Alexander,* W. Ralph Jones,* Alice B. Richards* and Benedict E. Abreau. Research Dept., Pitman-Moore Co., Indianapolis, Ind.

Narcotine produced bradycardia and vasodilatation in dogs anesthetized with sodium pentobarbital. Threshold effects were produced by single doses varying from 0.25 to 1.0 mg/kg while maximal responses were induced with single doses in the range from 2.5 to 5.0 mg/kg. Repetition of threshold doses at short intervals resulted in cumulation terminating in profound vasodepression comparable to that produced by single maximal doses. Acute tolerance to the bradycrotic and vasodilator effects appeared once this marked fall in blood pressure had occurred. Cross-tolerance to these vasodepressor effects was shown to exist between compound 48-80 and narcotine. Atropine, 1 mg/kg or bilateral mid-cervical vagotomy did not alter the vasodepression while essentially eliminating the bradycardia. Diphenhydramine, 15 mg/kg, eliminated the bradycardia and markedly reduced the vasodepression due to 5 mg/kg of narcotine. Bilateral carotid sinus and body denervation did not alter the narcotine cardiovascular effects. Narcotine, 2-4 μg/kg, injected into the circumflex coronary artery, increased coronary blood flow iwhtout altering other cardiovascular variables. Femoral arterial injections (25-50 μg/kg) increased limb blood flow without changing the systemic blood pressure; acute tolerance readily developed. Both narcotine and compound 48-80 increased bronchiolar resistance in anesthetized dogs. As in the vascular system, acute tolerance developed to bronchiolar constriction. The above data taken as a composite strongly suggest that many of the pharmacologic effects of narcotine are due to histamine liberation. Further, they demonstrate that the bradycardia is due to an as yet unexplained central effect.

1476. EXCRETORY PRODUCTS OF VARIOUS MERCURY COMPOUNDS IN ACIDOSIS AND ALKALOSIS. I. M. Weiner,* R. I. Levy* and G. H. Mudge. Dept. of Pharmacology, Johns Hopkins Univ. School of Medicine, Baltimore, Md.

Polarographic technique has been used to stody the mercury compounds excreted in the urine after the administration of mercuric chloride, p-chloromercuribenzoate and meralluride. All 3 compounds are excreted in combination with a sulfhydryl compound, probably cysteine. Since p-chloromercuribenzoate is not a diuretic, the loss of cysteine in the urine can not be implicated as the action of mercurials specifically responsible for diuresis. The urinary excretion of meralluride was studied during both acidosis and alkalosis. Since no consistent differences were found in these conditions, it is concluded that urinary excretion of the administered meralluride is not solely the determining factor in the diuretic response. After the administration of dimercaptopropanol (BAL) to dogs treated with meralluride, 2 new compounds appeared in the urine. One of these is the meralluride-BAL complex.

1477. EFFECTIVENESS OF IPRONIAZID ON 5-HYDROXYTRYPTAMINE (SEROTONIN) DESTRUCTION IN VIVO. Herbert Weissbach,* Donald F. Bogdanski,* Betty Redfield* and Sidney Udenfriend. Natl. Heart Inst., Bethesda, Md.

Monoamine oxidase (M.O.) is the major catalyst of serotonin metabolism in tissues and inhibitors of this enzyme are needed in studies on serotonin physiology. 1-Isopropyl-2-isonicotinyl-hydrazide (iproniazid), an effective inhibitor of M.O. in homogenates, has been considered effective in vivo because tissues removed from animals to which iproniazid had been administered, have little M.O. activity when homogenized. That iproniazid acts in vivo has been shown by its ability to potentiate the pharmacological actions of 5HTP, the precursor of serotonin, and to correlate this potentiation with increased amounts of brain serotonin. Further studies showed that although iproniazid administration produced 2-3 fold increases in endogenous brain serotonin levels, serotonin in other tissues was unaffected. Furthermore, iproniazid was found to have no effect on destruction of serotonin or tyramine when they were injected into animals and little effect on serotonin produced from 5HTP, except in brain. Since iproniazid treated animals, which were rapidly metabolizing serotonin, were found to be almost devoid of M.O. activity upon examination of tissue homogenates, it was concluded that homogenates were not a true index of the in vivo activity of this drug. Further studies revealed that slices prepared from tissues of iproniazid treated animals destroyed serotonin but that this activity was abolished by homogenization. It appears that except in brain, iproniazid does not penetrate to the site of M.O. while the cells are intact. Iproniazid is, therefore, an inefficient inhibitor of overall serotonin destruction but quite active in preventing serotonin metabolism in certain areas of the brain.

1478. MORPHINE AND MEPERIDINE AS RESPIRATORY DEPRESSANTS IN MAN. Herbert Wendel and Christian J. Lambertsen. Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.

Respiratory effects of 5, 10 and 15 mg/kg morphine sulfate and 50 and 100 mg/70 kg meperidine hydrochloride were studied in groups of 6-8 healthy men. Effects were measured at a constant "alveolar" pCO_2 of 46 mm Hg as changes in respiratory minute volume before and at intervals after i.m. drug injection. Plotting mean peak effect against molar dose of free base resulted in dose-effect curves virtually identical with theoretical curves drawn to the equation: Effect = 100/(1/(K [Dose]) + 1) which is based on dose-effect relations observed in isolated tissues. The results thus demonstrate that this dose-effect equation, expressing the mass action law, is also valid in man, at least for the respiratory effects of morphine and meperidine. Comparison of the 2 dose-effect curves reveals that 25, 56, 82 and 106 mg meperidine hydrochloride are as depressant to respiration as 5, 10, 15 and 20 mg morphine sulfate, respectively, indicating an average potency ratio of 5.4:1 for morphine to meperidine. Since the analgesic potency of morphine is 8-10 times that of meperidine, the therapeutic index (resp. depr. potency/analgesic potency) of meperidine is only about 0.6 that of morphine, and in equi-analgesic doses meperidine depresses respiration about 1.3 times as much as morphine.

1479. RESUSCITABILITY OF SEVERAL SPECIES OF ISOLATED MAMMALIAN HEARTS. Richard J. Wendel* and Theodore R. Sherrod. Dept. of

Pharmacology, Univ. of Illinois College of Medicine, Chicago.

In 1955 Sherrod reported that the isolated rabbit heart could be resuscitated following 1-2 hr of arrest at room temperature. Since that time, a comparative study has been made to determine the resuscitability of several species of isolated mammalian hearts, including the guinea pig heart, the cat heart, and the rabbit heart. This study has also determined the response of these hearts to ouabain following resuscitation. The hearts were perfused initially for 45 min with Ringer-Locke solution at 37°C and allowed to come to complete arrest following cessation of the perfusion fluid. The rabbit heart appeared to be most resistant to deprivation of the perfusion solution, taking an average of 22 min for complete auricular and ventricular arrest as compared with 12 min for the cat heart and 7 min for the guinea pig heart. These hearts were resuscitated after 1 hr of mechanical inactivity by reperfusion with Ringer-Locke solution. In each heart the ventricular beat was initiated within 2 min after restarting the perfusion. The guinea pig heart showed more heart block, ventricular irregularities and impaired force of contraction than did the rabbit or the cat heart. The response to ouabain was essentially the same for each heart, i.e., an increased force of contraction and a slowing of the heart rate. This effect was identical to that usually seen in such heart preparations prior to cardiac arrest. (Supported in part by a grant from the PHS.)

1480. ESTROGEN BIOSYNTHESIS FROM CHOLESTEROL-4-C^{14} AND ACETATE-1-C^{14} IN THE PREGNANT WOMAN. Harold Werbin, Jurgen Plotz, George V. LeRoy and M. Edward Davis (intr. by George T. Okita). Argonne Cancer Research Hosp. and Depts. of Biochemistry and Obstetrics and Gynecology, Univ. of Chicago, Chicago, Ill.

A woman in the fifth month of pregnancy, scheduled for therapeutic abortion, was given orally 87 uc of cholesterol-4-C^{14} (43.5 mg) in 2.0 ml of olive oil over a 6-day period. Six 24-hr urine samples were pooled and the steroid glucuronides were hydrolyzed with bacterial β-glucuronidase. Following continuous ether extraction of the urine, the estrogens were taken up in toluene and were extracted with 1 N NaOH by the procedure of Engel. The ketonic phenols were obtained by a Girard separation and the estrone and carrier were isolated by paper chromatography. The estrone was converted to the crystalline acetate derivative and counted twice. After hydrolysis of the acetate, the estrone was converted to the benzoate which was counted three times. Data will be presented to demonstrate the radiochemical purity of the isolated estrone. The counting was carried out with a liquid scintillation counter. Another pregnant woman was given i.v. 1.0 mc of sodium acetate-1-C^{14}. From a 3-day pool of urine radioactive estrone and estradiol were isolated. The estrone was radiochemically pure.

1481. PERSISTENCE OF RHYTHMIC PACEMAKER ACTIVITY DURING OUABAIN-INDUCED ASYSTOLE IN ISOLATED RABBIT ATRIA. Theodore C. West and Joseph V. Levy.* Dept. of Pharmacology, School of Medicine, Univ. of Washington, Seattle.

Effect of ouabain on sinus node action potentials has been studied in surviving rabbit atria using the microelectrode recording technique. At a concentration of 1:1.5 x 10^6, ouabain produced complete electrical and mechanical asystole of the atrial myocardium. Exploration of the sinus node region at this time, however, revealed that spontaneous rhythmic pacemaker activity was still present. Stimulation studies indicated marked depression of atrial excitability during this period. Washing the preparation with fresh Tyrode solution restored contractile tension and atrial cell action potentials. Under the conditions of these experiments, it appears that ouabain can preferentially depress excitability and conduction in the atrial myocardium without appreciably modifying pacemaker activity. (Supported by Natl. Heart Inst. Grant No. H-2213.)

1482. INFLUENCE OF AMBONESTYL ON OUABAIN-INDUCED CARDIOTOXICITY. Raymond H. Wheelock* and Byron B. Clark. Dept. of Pharmacology, Tufts Univ. School of Medicine, Boston, Mass.

Anti-arrhythmic action of ambonestyl in the coronary dog and during hypothermic cardiac surgery has been reported (Lanzoni and Clark. Circulation Res. 3: 335, 1955; Covino and Hegnauer. Surgery 40:475, 1956). Further experimentation was designed to test for similar activity during ouabain-induced ventricular tachycardia. Ambonestyl did not significantly affect the tachycardia but prevented fibrillation. Ouabain in a single i.v. dose of 0.110 mg/kg produced ventricular fibrillation in 72% of 18 unanesthetized dogs; in 12 additional animals in which the same dose of ouabain was followed 20 min later by ambonestyl (80 mg/kg) only 1 dog (8%) fibrillated. In order to elucidate a possible mechanism by which this anti-fibrillatory action is accomplished, the effects of ouabain, ambonestyl and a combination of both on ventricular excitability, refractory period and conduction velocity were determined in dogs with semi-closed chest under pentobarbital anesthesia using the general methods of Suckling et al. (Am. J. Physiol. 162:213, 1950) and Moe and Mendez (Circulation. 4:729, 1951). Ouabain, in nearly lethal cumulative doses, produced a decrease in excitability and conduction velocity and a shortening of the absolute refractory period. Ambonestyl (80 mg/kg) produced a lengthening of the absolute refractory period and an increase in conduction velocity with no significant alteration in excitability. Ambonestyl given before each increment of ouabain delayed all of the measured effects and increased the lethal dose of ouabain. A single dose of ambonestyl injected after a nearly lethal dose of ouabain produced a more rapid return of normal conduction velocity.

1483. COMPARATIVE RESPIRATORY DEPRESSANT EFFECTS OF MORPHINE, MEPERIDINE AND ALPHAPRODINE. Chester W. White, Jr., Robert Megirian* and Phillip S. Marcus.* Anesthesia Research Lab., Boston City Hosp., Boston, Mass.

Reported observations on narcotic respiratory depression have usually been on volunteer or anesthetized subjects. Methods of evaluation have differed widely; controlled data and statistical evaluations have been scanty. Clinical impression on this service concerning narcotic effects on respiration differed from accepted generalizations and somewhat from the literature. Accordingly, a study was carried out on approximately 50 postoperative patients who were representative of hospital cases to whom analgesics would normally be given. Either physiological saline or a narcotic was administered slowly i.v.; pulse, blood pressure, respiratory rate, inspiratory minute volume and reactiveness were recorded frequently during the subsequent 2 hr. Responses to saline were taken as control and effects of the narcotics were compared to them statistically. Ten patients receiving meperidine (1.5 mg/kg) showed significant depression of inspiratory volume (70-80% of controls) for 40' after administration; respiratory rate was

significantly altered (80-90% of controls) for 20'. Nine subjects receiving alphaprodine (0.38 mg/kg) showed a significant fall in inspired volume (60-90% of controls) for 20', but rate was unchanged. Nine subjects receiving alphaprodine (0.75 mg/kg) had significant depression of volume (50-80% of controls) for 25' and of rate (70-90% of controls) for 10'. Fifteen subjects receiving morphine (0.15 or 0.2 mg/kg) showed no significant change in ventilation volume or rate. No notable effects on pulse or blood pressure were seen. Thus, morphine is the least, meperidine intermediately and alphaprodine the most depressant to respiration of narcotics commonly used here. More detailed comparisons are contemplated.

1484. BLOOD CHOLINESTREASE STUDIES WITH SIMULTANEOUS ADMINISTRATION OF SEVERAL COMBINATIONS OF TWO ORGANIC PHOSPHATE INSECTICIDES. Martin W. Williams,* Henry N. Fuyat,* John P. Frawley and O. Garth Fitzhugh. Div. of Pharmacology, Food and Drug Admin., Dept. of Health, Education and Welfare, Washington, D. C.

This laboratory has observed pronounced potentiation in blood cholinesterase inhibition after simultaneous subacute administration of 0-ethyl, 0-p-nitrophenylbenzenethiophosphonate (EPN) and 0,0-dimethyl-S-(1,2-dicarbethoxyethyl) dithiophosphate (malathion). The present study was undertaken to determine the subacute effect on blood cholinesterase from the simultaneous administration of all possible combinations of two, at tolerance and safe levels in male and female dogs for the following compounds: 0,0-diethyl-S-ethylmercaptoethyl thiophosphate and 0,0-diethyl-0-ethylmercaptoethyl thiophosphate (demeton), 0,0-diethyl 0-p-nitrophenyl thiophosphate (parathion), 0,0-dimethyl 0-p-nitrophenyl thiophosphate (methyl Parathion), 0,0-dimethyl-S-(1,2-dicarbethoxyethyl) dithiophosphate (malathion), and 0-ethyl 0-p-nitrophenylbenzenethiophosphonate (EPN). The results of these experiments will be presented.

1485. INHIBITION OF GLYCOGENIC, MYOTROPHIC, AND ANDROGENIC ACTIVITY OF CERTAIN STEROIDS BY 2-AMINO-4-(p-CHLOROANILINO)-s-TRIAZINE IN THE RAT. Harold E. Williamson* and F. E. Shideman. Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison.

Capacity of 2-amino-4-(p-chloroanilino)-s-triazine to antagonize the effects of desoxycoritcosterone and 9 -fluorohydrocortisone on the urinary excretion of sodium and potassium by the rat has been reported (LeSher and Shideman, J. Pharmacol. & Exper. Therap. 116:38, 1956). The present study was undertaken to determine the effect of this triazine on the activity of certain glycogenic and myotrophic-androgenic steroids. For the measurement of glycogenic activity, the glycogen deposition assay of Pabst, Sheppard and Kuizenga (Endocrinology 41:55, 1947) was used. Adrenalectomized animals received either hydrocortisone (2.5 mg/kg) or 9 -fluorohydrocortisone (0.28 mg/kg) s.c. in 4 divided doses and triazine (5 mg/kg) was administered by i.p. injection. The hepatic glycogen deposition induced by hydrocortisone was decreased 45% and that resulting from 9 -fluorohydrocortisone was reduced 51% by administration of the triazine. The effect of the triazine on the myotrophic activity of two steroids was determined using the levator ani assay of Hershberger, Shipley and Meyer (Proc. Soc. Exper. Biol. & Med. 83:175, 1953). Castrated rats 21 days of age received testosterone propionate (0.05 mg) or 17-ethyl-19nortestosterone (0.2 mg) s.c. and/or the triazine derivative (0.1 mg) i.p. every day for a period of 1 wk. Administration of the triazine inhibited the increase in the weights of the levator ani muscles induced by testosterone propionate and 17-ethyl-19-nortestosterone 29% and 33%, respectively. The effect of the triazine on the increase in weights of the ventral prostate and seminal vesicles induced by the two steroids was utilized as a measure of its capacity to inhibit androgenic activity. This activity to be affected by the triazine only in the case of testosterone.

1486. URINARY EXCRETORY PRODUCTS OF ATROPINE. J. H. Wills, S. C. Kalser,* C. F. Epes* and J. D. Gabourel.* Pharmacology Branch, Directorate of Med. Research, Army Chem. Ctr., Md.

Using tropic acid or atropine, labeled with C^{14} in the alpha position of tropic acid, it has been found that tropic acid is excreted unchanged in the urine by the rat and the guinea pig, as had been found previously in the mouse and that atropine is not hydrolyzed to free tropic acid to any significant extent by the rat or the cat, as also had been found previously in the mouse. In the guinea pig, however, the major form in which the label from atropine appears in the urine is probably tropic acid. In both the rat and the cat the major excretory product of atropine is probably unchanged atropine. In the mouse it had been found previously that unchanged atropine is approximately equalled in importance as an excretory product after comparatively low doses of atropine by another unidentified compound having a rf of about 0.06 with a n-butanol: acetic acid:water solvent. This latter component probably appears also in the urines of the rat and the cat, but is much less important than in the mouse.

1487. GANGLIONIC STIMULATING AND PARASYMPATHOMIMETIC ACTIONS OF IMIDAZOLEPROPIONYLCHOLINE (DIHYDROMUREXINE) AND IMIDAZOLEACRYLCHOLINE (MUREXINE) IN CHLORALOSED DOGS. Martin M. Winbury, Joanne K. Wolf* and I. I. A. Tabachnick.* Dept. of Pharmacology, Research Div., Schering Corp., Bloomfield, N. J.

Imidazolepropionylcholine (ImPrCh) and Imidazoleacrylcholine (ImAcrCh) stimulate at two levels of the autonomic nervous system, i.e. at the ganglia and at the postganglionic parasympathetic effector sites. The ganglionic stimulation was observed on i.v. injection as a rise in arterial pressure which was blocked by hexamethonium. The peripheral parasympathomimetic action was observed on intra-arterial injection as a local vasodilatation which was blocked by atropine. Details follows. The i.v. actions of ImPrCh, 0.5-2.0 mg/kg, were similar to those of 1, 1 dimethyl-4-phenylpiperazinium (DMPP) or tetramethylammonium (TMA), 14 μg/kg, all three agents produced a rise in blood pressure (and blood flow) blocked by the same dose of hexamethonium, 2-3 mg/kg. Local intra-arterial injection of ImPrCh, 100-500 μg, or acetylcholine, 1-10 μg produced a prompt increase in the femoral blood flow; this was blocked by atropine, 1 mg/kg, i.v., but not by hexamethonium. DMPP and TMA, 200-500 μg intra-arterially also increased the blood flow, but this effect was blocked by hexamethonium. The vasodilatation produced by intra-arterial histamine, 1-10 μg, was not affected by atropine or hexamethonium. ImPrCh did not potentiate the intra-arterial vasodilator or i.v. hypotensive action of histamine.

1488. URINARY METABOLITES OF THIOPENTAL IN RAT, DOG AND MAN. Wallace D. Winters.

Dept. of Pharmacology, Marquette Med. School, Milwaukee, Wis.

Earlier reports from our laboratories revealed that desulfuration was a significant metabolic pathway of thiopental in a rat liver mince preparation. Other workers reported that thiopental carboxylic acid is the major metabolite of thiopental in man but not in the dog. The object of the present study was to clarify this apparent species difference. The urinary metabolites of thiopental were analyzed after paper chromtaographic separation in a butanol-NH system. The optical densities of the alkaline eluates were read at 225 mμ and 305 mμ to determine the rf values of the oxy- and thio- derivatives. The following rf values were obtained: 1) in the rat and dog, at 225 mμ, 0.06, 0.17, 0.32, 0.44, 0.69, 0.75, 0.92; at 305 mμ, 0.14, 0.30, 0.47, 0.56, 0.85; 2) in the human, at 255 mμ, 0.13, 0.32, and very small peaks at 0.66 and 0.92; at 305 mμ, 0.13, 0.29 and very small peaks at 0.59 and 0.81. Investigators studying the fate of pentobarbital have demonstrated that rf 0.46 contains the alcoholic derivative. Rf 0.09 corresponds to the carboxylic acid derivative, 0.75 to the parent compound and 0.14, 0.33, 0.69 and 0.93 to unidentified compounds. The thio derivative of thiopental with the rf at 0.14 corresponds to the carboxylic acid. Rf 0.56 has been postulated as the alcoholic derivative, 0.85 as the parent compound and 0.30 is unidentified. The major metabolite in man appears to be thiopental carboxylic acid and in dogs and rats the alcoholic derivatives of pentobarbital. (Supported by Public Health Service Grant B-912.)

1489. TOXICITY OF 5-FLUOROURACIL. T. Wong* and W. M. Benson. Dept. of Pharmacology, Hoffman La Roche, Inc., Nutley, N. J.

Among a group of fluorinated pyrimidines described by Heidelberger et al. (Fed. Proc., this issue), 5-fluorouracil has been found to possess significant anti-metabolic and tumor inhibiting activity in animals. Parenteral toxicity studies of 5-fluorouracil (Ro 2-9757) have been conducted in mice, rats, dogs and monkeys. Dogs are more susceptible to the toxic effect of Ro 2-9757 than other species. Deaths, when they occurred, were delayed irrespective of the species or dose. Repeated high s.c. doses of Ro 2-9757 (100 mg/kg in mice; 50 mg/kg in rats or 10 mg/kg in dogs) resulted in severe diarrhea, retardation of growth or loss of body weight. Hemorrhages were found in the stomach and intestinal contents were bloody. In some animals there were submucosal and subserosal hemorrhages of the intestinal tract and hemorrhages of the epi- and endocardial surfaces of the heart. Hemograms showed reductions of leucocytic and erythrocytic counts. The bone marrow elements in many of the animals were reduced; in others the medullary cavity showed varying degrees of hemorrhage. Slight changes were seen in mice given 25 mg/kg and in rats given 12.5 mg/kg for 14 days or in dogs given 2.5 mg/kg for 30 days. Preliminary studies in monkeys given Ro 2-9757 at levels several times the lethal dose for dogs showed no signs of toxicity. The toxic level of repeated daily doses of Ro 2-9757 in monkeys appears similar to that in mice and rats. Significant acute pharmacodynamic effects have not been demonstrated as yet. Preliminary comparative toxicity studies of 5-fluoroorotic acid have shown that it has toxic properties of the same order as 5-fluorouracil.

1490. EFFECTS OF NITRATE, IODIDE, BROMIDE AND SULFATE ON MAXIMAL ELECTROSHOCK SEIZURES (MES). Dixon M. Woodbury, Alan R. Koch* and N. K. Chakravarty.* Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.

The effects of nitrate, iodide, bromide and sulfate on MES in rats were tested because of the following observations: 1) nitrate, bromide and iodide inhibit carbonic anhydrase in vitro in concentrations which are approximately equal and which are also attainable in vivo whereas sulfate exerts no perceptible inhibition in concentrations attainable in vivo; 2) acetazolamide and sulfanilamide alter MES and this effect is directly related to the degree of carbonic anhydrase inhibition produced by these drugs. The ED_{50} for nitrate (peak time, 2 hr) for abolition of the tonic extensor phase of MES was found to be 9.6 mEq/kg; for bromide (peak time, 4-24 hr), 8.7 mEq/kg; for iodide (peak time, 4 hr), 9 mEq/kg. However, doses of iodide up to 17 mEq/kg still produced only 50% protection. Sulfate (4.7-19 mEq/kg) had no effect on MES. Groups of rats were allowed to drink solutions of $NaNO_3$, NaBr, or NaI for 8 days, the drugs were then withdrawn; after MES had returned to normal, the effect of CO_2 and acetazolemide on MES were tested. The rats of each group were not refractory to the anticonvulsant effects of both CO_2 and acetazoleamide. These data suggest that the effects of nitrate, bromide and iodide on MES can be explained by a single mechanism, namely, inhibition of brain carbonic anhydrase. (Supported in part by contract number 18(600)921, U.S. Air Force and grant B-381 from the Natl. Insts. of Health, PHS.)

1491. INFLUENCE OF COCAINE HCl UPON THE DEPRESSOR RESPONSE TO EPINEPHRINE. Robert A. Woodbury and Robert H. Shipp.* Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.

In dogs were adrenergic blockage has been established with piperoxan HCl or with phentolamine, cocaine HCl showed very limited capacity to restore the pressor response to epinephrine. In some but not all of these experiments cocaine prolonged the depressor response to epinephrine. The results are in contrast to those previously reported with ephedrine, hydroxyamphetamine, phenylpropylmethylamine and amphetamine, which did not prolong the depressor response to epinephrine. These results serve as additional evidence that cocaine potentiates the action of epinephrine by different mechanisms than those activated by ephedrine. (Supported by the American Heart Assoc.)

1492. ESTIMATION OF C^{14}-LABELED LEVORPHAN IN BIOLOGICAL MATERIALS. L. A. Woods. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

A specific and highly sensitive procedure has been developed for the estimation of N-methyl-C^{14}-labeled levorphan in biological materials. A 4.0-ml sample of diluted plasma, tissue homogenate, or urine, is mixed with 1.0 ml of a 0.1% aqueous solution of unlabeled levorphan (carrier), 4.0 ml of 40% aqueous Na_2HPO_4 and 10.0 ml of purified ethylene dichloride ($EtCl_2$). For the determination of total levorphan (free plus conjugated), samples are autoclaved with 1/10 volume of concentrated HCl and then neutralized with NaOH before the addition of the above reagents. The buffered mixture is shaken mechanically for 40 min, centrifuged, the aqueous layer removed and the $EtCl_2$ layer washed with 4.0% Na_2HPO_4. An 8.0 ml aliquot of the $EtCl_2$ layer is shaken with 5.0 ml of 0.5 N HCl and the $EtCl_2$ layer removed. The levorphan is re-extracted into $EtCl_2$ from an aliquot of the acid layer, which has been neutralized and buffered as indicated

above. An 8.0-ml aliquot of the $EtCl_2$ layer is slowly evaporated on an aluminum planchet using the barrel of a 10-ml syringe provided with a 1-1/4-inch no. 25 stainless steel needle fitted with a no. 25 stylet. The solid levorphan base is converted to the hydrochloride with alcoholic HCl and spread on the planchet as uniformly as possible with 50% methanol-ethanol. Radioactivity is determined in a gas-flow counter. Using buffer distribution studies the procedure has been shown to be specific for levorphan and can determine as little as 2-3 mμg of drug per ml or gm of sample. Recovery of added amounts of C^{14}-labeled drug is 105 ± 15% at the very low levels and 100 ± 6% at higher levels.

1493. EFFECT OF ASCORBIC ACID ON ANTIVIRAL ACTIVITY OF BIOFLAVONOIDS. S. Yoshimura,* L. S. D'Agrosa* and D. M. Green. Research Div., Grove Labs., Inc., St. Louis, Mo.

Groups of mice were infected with an LD_{100} dose of Influenza PR (a mouse-adapted human virus). All groups were then given, intragastrically, a minimally effective protective dose of a crude orange bioflavonoid complex twice daily for 5 days. The selected dose (100 mg/kg) was one found in other experiments to produce 5-20% survival. Ascorbic acid was concomitantly administered to certain groups, intragastrically, in doses which varied from 6.25 to 1600 mg/kg, depending on the group. The addition of ascorbic acid produced no consistent change in the protective effect shown by the crude bioflavonoid complex alone. The results failed to indicate that ascorbic acid synergized the antiviral effect of the bioflavonoid complex under the experimental conditions used.

1494. EFFECT OF CHLORAMBUCIL ON GROWTH AND METABOLISM OF YEAST (TORULOPSIS SP.). Richard S. Young* and Leon Hurwitz. Div. of Pharmacology, Food and Drug Admin., Washington, D. C.

Chlorambucil is a nitrogen mustard derivative that is presently being used as a carcinostatic agent. The purpose of this study is to elucidate a possible mechanism of action of this cytotoxic alkylating agent. It was felt that yeast would serve as a suitable organism for such a study. In actively growing cultures of yeast (Torulopsis sp.) this drug produces complete inhibition of growth in about 10^{-4} M concentrations. The inhibition of growth is less pronounced when citrate and glutamate are used as carbon and nitrogen sources than when glucose and ammonium chloride are used. The concentration of chlorambucil required to produce inhibition of growth will also inhibit the respiration and fermentation of glucose, while the respiration of citrate and glutamate together is much less sensitive. This decrease in sensitivity using citrate and glutamate as substrates is not due to the complexing of chlorambucil with these compounds. Our data suggest that inhibition of the growth of yeast by chlorambucil may be primarily due to the effect of this drug on some energy metabolism. At present the major site of action appears to be at some point other than the Krebs' cycle. (Supported by the Natl. Cancer Inst., PHS.)

1495. INHIBITORY EFFECT OF β-AMINOETHYLISOTHIURONIUM (AET) ON SULFHYDRYL ENZYMES. Gerald R. Zins* and Kenneth P. DuBois. U.S. A. F. Radiation Lab., and Depth. of Pharmacology, Univ. of Chicago, Chicago, Ill.

The demonstration by Doherty and Burnett (Proc. Soc. Exper. Biol. & Med. 89:312, 1955) of a protective effect by β-aminoethylisothiuronium (AET) against radiation lethality has stimulated interest in the pharmacological actions of this compound in animals. Recent studies by DiStefano et al. (J. Pharmacol. & Exper. Therap. 117:425, 1956) indicated that AET produces alterations in the blood pressure, inhibits the nictitating membrane and causes bradycardia, apnea and convulsions in intact cats. Since no information is available on the mechanism of the toxic effects of AET the present study was undertaken to ascertain the influence of this compound on the activity of various enzyme systems in vitro and in vivo. For these experiments aqueous solutions of the dichloride salt of AET were employed. The results of the study indicated that certain sulfhydryl enzymes are inhibited by AET. Thus the succinic dehydrogenase activity of rat liver exhibited a decrease from a normal Q_{O_2} of 78.8 to 41 by 1×10^{-3} M AET in vitro which represented 48% inhibition of the enzyme activity. Molar concentrations of 2×10^{-3} and 3×10^{-3} AET produced 60.6% and 68.1% inhibition, respectively, of rat liver succinic dehydrogenase and the enzyme activity of mouse liver exhibited similar susceptibility. The livers of mice killed 15-25 min after administration of AET i.p. exhibited inhibition of succinic dehydrogenase activity but the amount of inhibition was less than that produced by similar amounts of the drug in vitro.

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Forty-second Annual Meeting

CHICAGO, ILLINOIS, APRIL 15-19, 1957

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1496. **INCOMPATIBILITY REACTIONS IN HOMOLOGOUS GRAFTS EXPOSED IN VITRO TO X-IRRADIATION BEFORE IMPLANTATION.** Richard H. Andresen,* Clarence W. Monroe,* Dorothy A. Madden,* Fay H. Squire* and George M. Hass. Labs. of Pathology, Surgical Research and Radiology, Presbyterian-St. Luke's Hosp., Chicago, Ill.

Grafts of erector spinae muscle with attached fascia were resected from rabbits. Each graft was then exposed in vitro to a single dose of x-irradiation ranging from 100-90,000 r. Following this the tissues were implanted as autologous and homologous controlled grafts. Two weeks later, the animals were sacrificed and transplants prepared for microscopic study. At ranges of 10,000-90,000 r the tissue reaction was characterized by collagenous encapsulation of autologous and homologous grafts without vascular penetration. The basic architecture of grafts was unaltered. After 5,000 r, vascular penetration of the homograft occurred but this was unaccompanied by inflammatory infiltration, fibroblastic proliferation or collagen deposition. A similar pattern of reaction was noted in autografts. Inflammation did not follow vascularization of homografts unless the dosage was below 750 r. The action of irradiation in vitro failed to disclose any difference in the susceptibility of factors responsible for classical autologous and homologous host-graft interactions. The results indicate that classical homologous host-graft tissue reactions depend in part upon the magnitude of mutual admixture of cells and vascularized stroma from the 2 sources. The stimulus to admixture, though still unknown, is an important aspect of the problem.

1497. **CORRELATION BETWEEN SERUM TOTAL CHOLESTEROL AND CARDIOVASCULAR SUDANOPHILIA IN RATS FED CHOLESTEROL.** S. B. Andrus, L. C. Fillios*, D. M. Hegsted* and O. W. Portman.* Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

Fillios et al. (1956) reported from this laboratory the production of gross atherosclerosis in rats by feeding diets containing cholesterol, cholate and thiouracil. Since then a number of short-term studies have been made (8-17 wk), using lower levels of cholesterol and cholate and in the absence of thiouracil. Determinationf of serum total cholesterol were performed at intervals and at sacrifice, at which time the hearts and aortas were fixed in formalin and stained with Sudan IV. Sudanophilia was most prominent in the valvular endocardium, though aortic involvement was present in severely involved animals. The amount of cardiovascular Sudanophilia was graded objectively and correlated with the mean serum total cholesterol for each animal. This report comprises the accumulated experiences with these correlation data in 300 rats. Several different methods were employed for altering the basic cholesteremic response of the cholesterol-fed rat, including variations in the type of dietary fat, variations in the amount of cholesterol (0.45-4.0 gm) and cholate (0.15-1.35 gm), and various endocrine supplements in male, female and castrate animals. In all of these animals there was a positive correlation between cholesteremia and cardiovascular Sudanophilia ($r = +0.7$). Most lesions probably represent incipient atherosclerosis prior to the stage of intimal plaque formation, though the latter was seen microscopically in coronary arteries.

1498. **HISTAMINE TOLERANCE IN THYMECTOMIZED RATS AND GUINEA PIGS.** Ritsu N. Arison * and Herbert C. Stoerk. Merck Inst. for Therapeutic Research, Rahway, N. J.

Sackler et al. (1953) reported that thymectomy in rats is followed by an increased tolerance to histamine. This observation has been confirmed and extended in the rat. The histamine tolerance of guinea pigs, however, was not found to be modified by thymectomy. The modified tolerance to histamine was judged, as in previous experiments with other toxic agents (Nakashima & Stoerk, 1956), from the survival time of groups of animals repeatedly injected with a sublethal dose. Histamine dihydrochloride in 10% aqueous solution (500 mg/kg) was repeatedly injected intraperitoneally at 5-min intervals, and the number of injections given up to the occurrence of death was recorded. Symptoms appeared in greater severity in the sham-operated controls, and death ensued after a significantly fewer number of injections than in the thymectomized animals, the median survival time being 6 hr for thymectomized and 2 hr for controls. This contrast in tolerance was also demonstrable using smaller doses of histamine (2%) in rats which had been rendered sensitive to histamine by the administration of H-pertussis vaccine following thymectomy. The median survival time in these sensitized rats was 4 hr for thymectomized and 2 hr for sham-operated controls. Thymectomized guinea pigs did not exhibit enhanced resistance, the median survival time being 20 min for thymectomized and 35 min for sham-operated controls.

No difference was observed between thymectomized and sham-operated rats in their sensitivity to curare, acetyl choline or physostigmine administered in repeated doses.

1499. PRODUCTION OF EPITHELIAL INCLUSION CYSTS BY AUTOGENOUS TRANSPLANTATION OF PURE EPIDERMIS IN MICE. Charles A. Ashley and Lore Robinson.* Mary Imogene Bassett Hosp., Cooperstown, N. Y.

A method of autogenous transplantation of epidermis, free of native stroma, to various internal sites was developed. This allows observation of the effects of various physical and chemical agents applied either prior to or in the course of the operative procedure, while minimizing the possible effects of such agents on supporting tissues. Triangular Thiersch grafts (4 x 4 x 3 mm) were cut from the ears of mice anesthetized with intraperitoneal pentobarbital. The epidermal surfaces were then attached to waterproof tape along 2 edges. Grafts were covered with a sterile solution of 0.5% commercial Trypsin in Krebs-Ringer-Phosphate solution (pH 7.3). Incubation at 37°C resulted in separation so that the dermis could be removed easily. Sheets of epidermis without supporting connective tissues remained attached to the tape. Using fine jeweler's forceps, the central unattached portion of the epidermis was transplanted autogenously into deep fascia, skeletal muscle, kidney and liver. Grafts placed between the panniculus carnosus and deep fascia of the mid-dorsal region were most satisfactory. This technique was used in one series of mice which were sacrificed at varying time intervals. The grafts were excised and studied histologically by serial step sections. The results were compared with a second series in which complete Thiersch grafts were similarly transplanted. Surviving grafts usually organized into rounded cysts. These contained a central area of necrotic and desquamated cornified cells with peripheral layers of viable epithelial cells showing rare intercellular bridges. The line of demarcation from adjacent connective tissue was irregular and indistinct.

1500. USE OF ORGAN CULTURES IN STUDY OF INFECTION. Frederik B. Bang and Janet Niven.* Johns Hopkins Univ., Baltimore, Md., and Natl. Inst. for Med. Research, London, England.

Using recent modifications of the watch glass technique (Schafer. Expt. Cell Res. 11:1, 1956) human embryonic tracheal, nasal mucosa and adult bronchial mucosa were grown on chicken plasma clot as organ cultures. They were maintained for periods up to 2 months with continued mitosis. The epithelial cells remained ciliated, mucous glands and cells continued to secrete mucous. Several strains of Newcastle disease virus were grown in chick tracheal cultures and the lesions so produced studied. Organ cultures of adult ferret nasal mucosa were inoculated with influenza virus and extensive destruction of the superficial epithelium produced. This effect was neutralized by convalescent ferret antisera. Human embryonic skin cultures remained organized and spread over the supporting rayon net. Vaccinia and herpes viruses produced characteristic lesions on these cultures.

1501. ERYTHROCYTE PYRIDINE NUCLEOTIDES. Harry N. Barnet (intr. by Howard A. Ball). Research Dept., San Diego Blood Bank, San Diego, Calif.

Quantative ion-exchange chromatographic techniques for the isolation of human erythrocyte pyridine nucleotides have been developed for metabolic studies of these compounds in normal fresh and stored blood. Diphosphophridine nucleotide (DPN) and nicotinaminde mononucleotide (NMN) are first quantatively isolated and separated from the red cell (RBC) extract (trichloroacetic acid) on Dowex 50 cation exchange resin by elution with 0.003 N HCl and water respectively. The neutralized sample eluant containing the more acidic triphosphopyridine nucleotide (TPN) is then passed through a column of Dowex 1 anion exchange resin and eluted with 0.01 N HCl. Isolation of the acid labile reduced DPN (DPNH), and TPN (TPNH) is made from a separate hot water extract of RBC's on a neutral Dowex 1 column by elution with neutral salts or buffers. Using these techniques the levels of the pyridine-nucleotides in fresh and stored erythrocytes has been established. The DPN concentration in normal fresh human erythrocytes has been found to be 30-37 g/ml RBC's (4.5-5.5 μM/100 ml RBC's) while in blood stored 21 days at 4-10°C the level was 20-25 μg/ml RBC, a loss of approximately 20%. The levels of NMN and TPN were found to be essentially unchanged after 21 days' storage being 5-7 and 8-12 μg/ml RBC respectively.

1502. PAPER CHROMATOGRAPHIC PATTERNS OF HUMAN VAGINAL FLUID. W. M. Berton (intro. by D. H. Sprunt). Inst. of Pathology, Univ. of Tennessee, Memphis.

Crude human vaginal fluid was obtained from clinic patients and chromatogrammed on Whatman No. 1 chromatographic filter paper following modifications of the technique as described by Rogers and Berton. The resultant chromatograms were visually and photometrically analyzed with incandescent and ultraviolet light sources, the latter of the 2540°u. and 3660°u. range. An attempt was made to use only the most simply prepared solvents and developing agents. Consistently reproducible chromatograms were observed when the material from a single patient was positioned at separate points along the base line. Similarly, consistently reproducible individual differences, both in the ninhydrin positive and fluorescent patterns, were noted when material from a sampling of clinic patients was simultaneously chromatogrammed. An attempt to correlate individual chromatographic variations with concurrent cytologic and/or histologic diagnosis was made. The results of such a correlation were most interesting in those cases of intraepithelial neoplasia of the cervix uteri. It would appear that there is a decrease to absence of a high ninhydrin positive component in addition to changes in the lower ultraviolet positive materials, especially those fluorescing within the 2540°u. range. The chromatographic pattern of the secretions from the pregnant female with suspicious cytologic smear was investigated as such materials became available. Initially, it would appear that complicating factors, such as the menstrual cycle, T. vaginalis infestation, the presence of small amounts of blood in the vaginal fluid, and the menopause, do little to alter basal chromatographic patterns.

1503. EFFECT OF DEHYDROCHOLIC ACID ON EXPERIMENTAL CHOLELITHIASIS IN THE RABBIT. M. Bevans, R. Kaplan* and E. H. Mosbach.* Columbia Univ. Research Service, Goldwater Mem. Hosp., and Dept. of Medicien, Columbia Univ., New York City.

It has previously been demonstrated that rabbits receiving diets containing 1% dihydrocholesterol for 3-8 wk consistently develop biliary calculi and inflammatory lesions of the biliary tract. The present studies are concerned with the effect of dehydrocholic acid (3, 7, 12-triketocholanic acid), a hydrocholeretic agent, on the development of the dihydrocholesterol-induced

lesions. It was found that dehydrocholic acid partially or completely prevented the development of experimental cholecystitis and cholelithiasis when it was administered simultaneously with the sterol. The effect obtained depended upon the relative concentrations of dihydrocholesterol and dehydrocholic acid in the diet. Dehydrocholic acid did not prevent the absorption of dihydrocholesterol from the intestinal tract since tissue storage of this sterol was increased in animals receiving diets containing dihydrocholesterol plus dehydrocholic acid. Additional experiments were carried out designed to evaluate the curative effect of the choleretic agent administered to rabbits in which cholelithiasis already existed. Administration of the choleretic agent appeared to have no effect in causing a regression of the gross biliary lesions after these had already become established. Possible mechanisms underlying these observations will be discussed.

1504. COMA OF ECK'S FISTULA ANIMALS ASSOCIATED WITH INCREASED FREE AMINO ACIDS OF THE BRAIN AND SPINAL FLUID. J. L. Bollman, E. V. Flock,* J. H. Grindlay* and F. R. Lichtenheld.* Mayo Fndns. and Clinic, Rochester, Minn.

If all of the portal blood of the dog is diverted from the liver by portal caval anastomosis, the animal will progressively lose weight and die in less than 1 yr. Before termination one or more periods of characteristic coma will ensue. Electro-encephalographic tracings indicate rapid waves suggesting a convulsive type of seizure, different from the slowed waves of clinical hepatic coma and those which occur following complete removal of the liver. During periods of symptoms the free amino acid content of the spinal fluid may be considerably elevated and increases are found in the free amino acids of the brain. The free amino acid content of the blood is usually not elevated. The occurrence, intensity and duration of symptoms do not appear to be directly related to the magnitude of increase of the free amino acids of the brain or spinal fluid. Glutamine is present in spinal fluid in larger amounts than any single amino acid and is increased more. The glutamic acid content of the brain is not decreased, when the glutamine content is increased. Partition of the free amino acids by paper chromatography did not indicate that any one of the amino acids studied was specifically elevated more than in proportion to others. None of the single amino acids studied could be directly correlated with the intensity or duration of symptoms.

1505. CELLULAR BASIS OF ANTIBODY FORMATION: DEVELOPMENT DURING NEONATAL PERIOD. Robert A. Bridges,* Richard M. Condie,* Solomon J. Zak* and Robert A. Good. Dept. of Pediatrics, Univ. of Minnesota, Minneapolis.

Studies were carried out along 3 lines. 1) Serial morphologic, immunologic, electrophoretic and immunochemical studies were made on an apparently normal child born of an agammaglobulinemic mother. The neonate was agammaglobulinemic, immunologically unresponsive and failed to form plasma cells after antigenic stimulation. Beginning 42 days after birth, gamma globulin and specific antibodies appeared in the serum. Morphological studies in the immediate neonatal period revealed absence of plasma cells from bone marrow and lymph nodes and failure of plasma cell development in response to antigenic stimulation. At 3 months when antigenic stimulation resulted in antibody production, plasma cells were present in the marrow and developed in the regional lymph nodes. At 14 months the baby had normal amounts of gamma globulin and formed antibodies, plasma cells and secondary follicles. 2) In normal human newborns, plasma cells were absent from bone marrow. The serum showed a logarithmic decline of gamma globulin concentration suggesting failure of gamma globulin formation. Necropsy studies of submucosa from ileum and appendix revealed absence of plasma cells from bowel of premature and newborn infants and gradual appearance of plasma cells beginning 4-6 wk post natal. 3) Finally, newborn rabbits failed to develop plasma cells and antibody following antigenic stimulation whereas 28-day-old rabbits stimulated with the same antigen formed both antibody and plasma cells. These data indicate that human and rabbit newborns are immunologically null and that the morphological basis for this inadequacy is failure of plasma cell development. (Aided by grants from the PHS, Helen Hay Whitney Fndn., Minnesota Heart Assoc. and American Heart Assoc.)

1506. STANDARDIZATION OF ATP-ASE ACTIVITY IN ISOLATED CARDIAC MYOFIBRILS. Arnold Brown,* Albert Aras* and George Hass. Rush Lab. of Pathology, Presbyterian-St. Luke's Hosp. Chicago, Ill.

A method has been developed for isolation of large quantities of cardiac myofibrils which retain their contractile properties. Twenty to 40 gm of canine or human myocardium are placed in a colloid mill with a glycerol:0.1 M Tris buffer, pH 7.0 (4:1) substrate and milled for 60-90 min. Following this the suspension is purified by differential centrifugation at 0° C. The final suspension consists of a 20-30% yield of single myofibrils in which the characteristic band structure, as revealed by light and electron microscopy, is retained. Under suitable conditions the addition of ATP to the suspension results in prompt complete contraction of the myofibrils. ATP-ase activity of purified myofibril suspensions was determined by measuring inorganic phosphate accumulation in supernatants following addition of ATP. This activity varied with concentration of ATP and pH in myofibril suspensions to which 0.1 mM of Mg^{++} and Ca^{++} had been added. Maximal ATP-ase activity at pH 7 occurred at an ATP concentration of 1.0 mM with either ion. The amount of inorganic phosphate liberated in 2 min was a linear function of myofibril concentration. Storage of heart muscle at room temperature for 6 hr and then at 4° C for 18 hr before making the myofibril preparations resulted in only a 5-10% loss of ATP-ase activity. This has permitted comparative analyses of human myocardiums obtained in the customary postmortem period. The procedure is equally suitable for skeletal myofibrils.

1507. CORRELATION OF CHEMICAL AND PATHOLOGICAL CHANGES IN EXPERIMENTAL AMYLOIDOSIS. E. Calkins, R. G. Giles and A. S. Cohen (intr. by B. Castleman). Dept. of Medicine, Harvard Med. School, and Med. Services, Massachusetts General Hosp., Boston.

Longitudinal studies of rabbits during the induction of amyloidosis with casein have been undertaken for 2 purposes: a) to differentiate by simple means those animals which develop amyloidosis from those which do not and b) to define clinical course and, if possible, clarify the pathogenesis of this disease. Various protocols were followed: 16 rabbits received a suspension of sodium caseinate subcutaneously twice weekly from 5 - 15 months (15 developed significant amyloidosis); 3 rabbits received only 1 s.c. injection and were subsequently followed for 8 months (1 developed massive amyloidosis); 3 rabbits received no

injections and did not develop the disease. Serial determination of serum proteins by paper electrophoresis estimations of antibodies to casein, and determination of serum hexosamine by NPN were performed at 1- to 2-month intervals. Repeated spleen biopsies were performed on nearly one half of the rabbits. Most animals were sacrificed by exsanguination. Livers, spleens, kidneys, hearts and injection sites were studied histologically. Results showed that those animals which, early in the course of injections, developed significant elevations in their serum globulins and hexosamine concentrations were those which went on to develop amyloidosis. Significant titers of circulating antibodies were demonstrable in many rabbits but their presence or absence bore no relationship to the eventual development of the disease. I^{131} labeled caseinate, administered to 3 amyloid animals prior to sacrifice resulted in no concentration of I^{131} in the spleens. Other methods of inducing amyloidosis were similarly studied, and the results compared to the above. The relationship of the date to current theories of the genesis of amyloidosis will be discussed.

1508. BASIC PROTEIN IN CYTOPLASM OF EHRLICH ASCITES TUMOR CELLS. W. H. Carnes and B. R. Forker.* Depts. of Pathology, Stanford Univ. School of Medicine, San Francisco, Calif., and Univ. of Utah College of Medicine, Salt Lake City.

The biological role of histones is generally inferred to be related to that of DNA owing to their firm bond in isolated nucleoprotein, their identical nuclear localization (Alfert and Geschwind. Proc. Nat. Acad. Sci. 39: 991, 1953), and their proportionate synthesis during mitosis (Block and Godman. J. Biophys. Cytol. 1:17, 1955). A similar relationship of basic protein to RNA in the cytoplasm of ascites tumor cells is suggested by the binding of Fast green at pH 8 after fixation in formalin or Carnoy's fluid. Like the strong basic groups of nuclear histone those of the cytoplasmic protein are masked by the nucleic acid and bind the dye only after extraction of RNA with trichloracetic acid or ribonuclease. The Feulgen reaction shows no DNA in the cytoplasm of these cells. The distribution and relative concentration of Fast green bound are directly related to the cytoplasmic RNA concentration as judged visually by its basophilia. Preliminary survey of normal mouse and human tissues has shown smaller amounts of Fast green bound by the cytoplasm of certain other cells (e.g. pancreatic acinar cells, neurons) with the same relationship to RNA. The significance of the higher concentration of this type of protein in the ascites tumor cells remains to be explored. (Supported by PHS research grant C-1738(C3) and the Am. Cancer Soc. grant INSTR-78B.)

1509. DISSIMILARITY IN O_2 CONSUMPTION OF THE THORACIC AND ABDOMINAL AORTA. R. W. Christie* and L. K. Dahl. Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Respiration of the thoracic and abdominal portions of the rat aorta has been measured by the Warburg technique as part of a study on the effects of prolonged Na^+ ingestion. Two groups of rats which were fed the same basic diet containing 0.2% NaCl have been compared; 16 animals received 1% NaCl in their drinking H_2O for more than 9 months while the group of 14 control animals received only H_2O. The thoracic segments were found to respire significantly more actively than the abdominal segments in both groups. $Q_{O_2}^{O_2}$(N) for thoracic - 12.6±4.2; abdominal 8.5±3.3, $p << .01$. The rates were not significantly different among animals on or off added NaCl. There were no histological differences in the 2 segments. However, we have obtained suggestive but not conslusive evidence that in young animals the 2 aortic segments respire at similar rates; furthermore, there appears to be a decline in the O_2 consumption by the abdominal segment as the animal ages. In view of the known predilection of the abdominal aorta to atherosclerosis, these findings warrant further investigation for it seems possible that the sites of the atherosclerotic process may be conditioned by a lower metabolic rate at such site, prior to the inception of the process. (Supported by the Atomic Energy Comm.)

1510. LOCUS OF ABSORPTION OF VITAMIN B_{12}. Yale Citrin,* Carlo DeRosa* and James A. Halsted. VA Hosp., and Dept. of Medicine, State Univ. of New York, College of Medicine, Syracuse.

In subjects with and without pernicious anemia, physiologic doses (0.5-1.5μg) of $Co^{60}B_{12}$ was delivered by intestinal intubation to various levels of the intestinal tract. Using 2 flushing doses, absorption was assessed by the urinary excretion test, urine being collected for 48 hr. Good reproducibility in the same individual was demonstrated. When $Co^{60}B_{12}$ was delivered to the duodenum of 5 normal subjects, from 15.6-39.5% was recovered. In 4, recovery following duodenal instillation was significantly greater than after oral administration. After jejunal instillation, 12.3, 22.3 and 22.8% recovery, respectively, occurred in 3 subjects. In 6 subjects, the test dose, delivered to the lower ileum, resulted in urinary recoveries of 0, 0, 4.7, 16.5, 18.4 and 23.2%, respectively. In 4 patients with pernicious anemia, the test dose, to which was added a potent intrinsic factor preparation, was delivered to the lower ileum. Significant absorption occurred in all. Without added intrinsic factor, no absorption was demonstrable. Fourteen test doses, alone, with intrinsic factor, or after Neomycin sterilization, instilled into the sigmoid of normal subjects, and 1 dose, with intrinsic factor, instilled into the transverse colon, resulted in no absorption. These results suggest that the lower small intestine, but not the large bowel, is capable of absorption of B_{12} in physiologic amounts; and that absorption from the ileum requires the presence of intrinsic factor.

1511. USE OF VITAL STAINING IN EXPERIMENTAL CEREBRAL EDEMA. Raymond A. Clasen,* Pauline Cooke,* Felix Martin,* Anthony Raimondi* and George M. Hass. Lab. of Pathology, Presbyterian-St. Luke's Hosp., Chicago, Ill.

Following intravenous Evan's Blue or methylene blue, acute closed cerebral lesions were produced in dogs by freezing with liquid air. The animals were sacrificed at varying periods. In the Evan's Blue group gross examination revealed the following. Five minutes after production of a lesion the damaged area was defined by a slight increase in blue color. After 1 hr the lesion area was intensely stained but there was no staining of the adjacent white matter. After 4 hr both the lesion area and adjacent white matter were stained. Following use of methylene blue the lesion failed to stain, although the rest of the gray matter was colored intensely. There was no difference in the color of the white matter adjacent to the lesion and that of the opposite hemisphere. Microscopically, the lesion area showed acute necrosis and perivascular hemorrhage. Those areas stained by Evan's Blue after 4 hr appeared as foci of rarefaction in the white matter microscopically. With the Kluver-Shiff stain such areas contained granular pink material. The myelin sheaths were swollen. Sections from the methylene blue group failed to reveal axonal degeneration. The distribution of the Evan's Blue staining-Shiff positive zones was not entirely related to their proximity to the lesion

area since some fiber groups even adjacent to the lesion were normal. The connections between these findings, acute neuronal degeneration and cerebral edema, as defined by quantitative chemical analyses of water and electrolytes, will be discussed.

1512. EFFECTS OF PITUITARY, GONADS AND ADRENALS ON GROWTH AND FUNCTION OF AUTONOMOUS MAMMOTROPIC TUMOR CELLS IN RATS. Kelly H. Clifton* and Jacob Furth. Children's Cancer Research Fndn. Children's Med. Ctr., and Dept. of Pathology, Harvard Med. School, Boston, Mass.

Tumor growth was retarded by gonadectomy of hosts of either sex and was enhanced by estrogen in direct proportion to the quantity of hormone given. Growth was slightly increased by a single pellet containing 0.01 mg stilbestrol, more markedly by 0.10 mg, and greatly by 1.0 mg. Growth of tumors in hypophysectomized, diethylstilbestrol-treated animals was nearly as great as in estrogen-treated unoperated hosts, and greater than in gonadectomized animals. Adrenalectomy did not markedly alter the rate of mammotropic tumor growth. These mammotropes caused full development of mammary glands with milk secretion and an increase in weights of the body, liver, spleen, heart, kidneys and adrenals. These changes occurred in gonadectomized hosts without estrogen but were much enhanced by this hormone. Other pituitary hormones were unnecessary for these mammosomatotropic effects since they occurred in estrogen-treated hypophysectomized hosts. Adrenalectomy, however, prevented milk secretion but not proliferation of the epithelium of the mammary glands. Estrogen at the dosage used had but slight effect on the mammary gland in absence of mammotropin. It is postulated that estrogen acts on the mammary gland mainly by stimulation of pituitary mammotropes.

1513. NEPHRITIS INDUCED BY THORIUM^{228} CITRATE IN DOGS. Terrence H. Cochran,* James S. Arnold and Webster S. S. Jee.* Radiobiology Lab. and Depts. of Anatomy and Pathology, Univ. of Utah College of Medicine, Salt Lake City.

Radiothorium (TH^{228}) prepared in a citrate buffer of pH 3-3.5 and injected intravenously into dogs produced marked damage to the kidneys. The dose level and duration of survival were critical for the production of the severe nephritis. The most marked changes occurred in those animals receiving 2.5 μc/kg and surviving approximately 200 days. Those animals receiving the same dose and surviving less than 100 days showed only minimal changes. Animals receiving 0.81 μc/kg and surviving 600-800 days showed moderate changes. Animals receiving 0.27 μc/kg and surviving up to 1000 days showed only minimal tubular changes. Thorium^{228} citrate excretion in dogs differs from the other isotopes studied (plutonium, Pu^{239}, and radiu, Ra^{226}) in that the greater portion is excreted by the kidneys and at sacrifice there is a much higher percentage retention in the kidneys (approximately 1% of the injected dose). Radioautographs show a high concentration of the thorium^{228} in the glomeruli and also in the blood vessels throughout the body.

1514. IN VITRO RESPONSE TO ACTH OF FUNCTIONAL EXPERIMENTAL ADRENOCORTICAL TUMORS. Arthur I. Cohen and Eric Block (intr. by Jacob Furth). Children's Cancer Research Fndn., Children's Med. Ctr., Boston, and Worcester Fndn. for Exptl., Biology, Shrewsbury, Mass.

Slices from transplants of 2 secretory adrenocortical tumors, one originating in an irradiated mouse, and the other in a radiothyroidectomized rat, were incubated with and without ACTH. The total Δ 4-3-ketosteroids produced were extracted and quantitatively determined from peak optical densities at 242 m . The basal corticoid secretion in the mouse and rat tumor slices averaged about 2.6 and 1.2 μg equivalents of cortisol/100 mg/2 hr; with maximal stimulating amounts of ACTH, the rates were 13.6 and 13.1 μg, respectively. Both tumors responded to ACTH levels as low as 0.1-0.5 mU; maximal stimulation occurred at 2-3 mU of the hormone. Under ACTH stimulation, corticosterone was the major steroid synthesized by normal mouse adrenal incubates (72% of total Δ4-3-ketosteroids); 11β-hydroxy-androstene-3, 17-dione was present in small quantities. Mouse adrenal tumor slices incubated with ACTH produced approximately equal amounts of these 2 steroids. However, 25-65% of total Δ4-3-ketosteroids consisted of compounds with chromatographic behavior suggestive of progesterone, 11β-hydroxyprogesterone, 11-dehydrocorticosterone and Δ4-3-keto-20,21-dehydroxy steroids. Bioassays of these tumors failed to indicate secretion of gonadal hormones.

1515. CAUSE OF DEATH IN SUBLETHALLY IRRADIATED MICE GIVEN RAT BONE MARROW. C. C. Congdon, T. Makinodan* and N. Gengozian.* Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

The 30-day mortality in 198 C_3H X 101 F, hybrid mice exposed to 710 r of x-radiation was 30%. Forty-one mice similarly irradiated but given rat bone marrow intravenously afterward showed 100% mortality in 16 days. Fifty mice given 710 r and isologous bone marrow had no deaths in the 30-day period. At necropsy the mice that died after rat bone marrow treatment showed bone marrow aplasia as well as hemmorrhage, infection and anemia. A serial killing experiment was performed in which 4 groups of 20 mice each were killed and necropsied during a 30-day period. The groups were 710 r plus rat bone marrow, 710 r only, 710 r plus isologous bone marrow, and unirradiated mice plus rat bone marrow. Irradiated mice that received isologous or heterologous bone marrow showed recovery of bone marrow and splenic red pulp in 5 days. The untreated irradiated mice recovered in 2 wk. The unirradiated mice injected with rat bone marrow showed no dramatic changes. Complete secondary destruction of the bone marrow and splenic red pulp was observed 6-8 days after exposure in the irradiated mice given rat bone marrow. In this group, the spleen and lymph nodes showed marked epitheloid cell response concomitant with the disappearance of the blood-forming tissues. The secondary destruction of the blood-forming tissues in mice given 710 r and rat bone marrow lead to the death of the mice through hemorrhage, infection and anemia. To further test this hypothesis, 20 mice were exposed to 710 r and given rat bone marrow. Six days later, each received a massive dose of isologous bone marrow. Six days later, each received a massive dose of isologous bone marrow intravenously. Four mice died in 15 days and 16 recovered.

1516. SPECIFICITY OF MANGANESE PATHWAY IN THE BODY. George C. Cotzias and James J. Greenough.* Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Mice maintained on Purina Fox chow were injected with .2 μc Mn^{54} Cl intraperitoneally. They were then placed in a Texaco counter daily for 10-30 days to determine the radioactivity retained. From this time turnover rate was calculated. In semilogarithmic plots, 2 distinct such rates were encountered, with a half-life of 48-68 hr and 232-304 hr respectively. A

manganese-poor diet increased these numbers, while manganese supplements decreased them in rough proportion to the concentration of the metal in the diet. Injections of manganous, manganic and permanganic salts also markedly accelerated the rate of loss of $Mn^{54}SO_4$. This demonstrated that a large, freely exchangeable fraction of radiomanganese existed in the body throughout these experiments. In other experiments, instead of loading these tagged animals with natural manganese salts, other members of the transition-group were used. Bivalent salts of vanadium, chromium, iron, cobalt, nickel, copper and zinc were injected or fed in doses approaching the border of toxicity. There was no effect whatsoever on the turnover of manganese, irrespectively as to whether experimental animals were compared to controls or to their own preinjection turnover rates. Similarly, administration of members of group VII of the elements in the form of sodium bormide, sodium iodide and sodium perrhenate failed to affect the normal turnover rate of radiomanganese. From this it is concluded that there exists in the body a manganese pathway of high specificity for this metal. (Supported by the Atomic Energy Comm.)

1517. HEMATOLOGIC EFFECTS OF FURADROXYL IN DOGS. Robert D. Coye (intr. by D. Murray Angevine). Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.

Furadroxyl, a chemical compound of the nitrofuran group, has previously been shown to cause reversible inhibition of spermatogenesis and other members of this group have been shown to have inhibitory effects on transplanted fibrosarcoma and bacterial growth. Recent investigations have shown furadroxyl to cause bone marrow and testicular aplasia in dogs. In the work reported here severe aplastic changes were induced in the bone marrow of dogs fed furadroxyl. Effective doses were in the range of 50 mg/kg/day for a minimum of 3 wk. The course of the toxicity was followed by peripheral blood studies, bone marrow biopsies and autopsy examination. A fall in the platelet count and the appearance of giant platelets was the first detectable response; this was followed by leukopenia with a fall in the number of both granulocytes and lymphocytes, and later a slight fall in the hemoglobin level. Of the 5 dogs used, 2 recovered and 3 died of severe marrow aplasia. Biopsies and autopsies showed only thiny focal areas of hematopoietic tissue remaining in a loose reticulum.

1518. CHRONIC REMITTENT DERMAL LESIONS IN RABBITS FOLLOWING A SINGLE INJECTION OF AN EXTRACT OF GROUP A STREPTOCOCCI. William J. Cromartie and John H. Schwab.* Depts. of Bacteriology and Medicine, Univ. of North Carolina, Chapel Hill.

A sterile sonic extract of group A, type 3, streptococci (Strain D-58) was prepared using the method previously reported by Schwab (J. Bact. 71:94, 1956). Thirty New Zealand white rabbits, each weighing 2000-2500 gm, were injected intradermally at a single site with 0.2 ml of this material. Two to 5 days after injection all animals developed nodulations of the skin at and around the site of injection in an area that varied from 50 x 30 to 120 x 80 mm. The separate nodules varied in size and shape from rounded elevations 8 x 8 mm to irregular elongated ridges 15 x 50 mm that projected 6-12 mm above the skin surface. The reaction has been observed to continue for at least 30 days and was characterized by the gradual disappearance of nodules and the development of new nodules as late as 28 days after injection. The gross and microscopic changes will be illustrated. The most marked changes occurred deep in the dermis and were associated with "fibrinoid change" in the connective tissue and a variety of cellular reactions including diffuse infiltration with polymorphonuclear cells, lesions suggesting abscess formation, and focal granulomatous lesions containing many giant cells. The injection of appropriate control material, including similar extracts of streptococci other than group A, produced no comparable lesions. Comparable lesions have been produced with extracts of other types of group A streptococci.

1519. TOXICITY OF ANTIRABBIT RED CELL HEMOLYTIC SERUMS PRODUCED IN GUINEA PIGS FOR THE SKIN OF RABBITS. Israel Davidsohn, Raymond Muratore,* Eleanor G. Hanawalt* and Israel S. Goldman.* Chicago Med. School and Mount Sinai Med. Research Fndn., Chicago, Ill.

Hemolytic serums produced as stated in title were capable of producing acute fatal shock, acute fatal hemolytic or moderate anemia, with recovery in the rabbit after intravenous, intramuscular or intracutaneous injection. The general systemic effect was dependent on the hemolytic potency of the serum, dose and route. The intravenous route was the most effective. The dosage needed for each of the 3 clinical forms was established for each lot of serum. The preceding observations were reported previously (Davidsohn et al. Proceedings, 6th Congress of Inter. Soc. of Hematology, 1956). New observations dealing with changes following i.c. injections will be reported. The i.c. route was followed by rapidly developing, severe local changes with swelling, redness, necrosis and sloughing. Microscopically, massive accumulation of neutrophilic granulocytes and destruction of tissue were the main findings. The intensity of the effect was directly proportionate to the amount of serum used. The outcome ranged from complete restitution of the site of injection to formation of an abscess, falling off of slough, emptying of purulent contents, with subsequent healing. Injection of normal guinea pig serum was followed by transient, slight local redness and occasionally by swelling, both disappearing in a matter of hours or days, depending on the dose used. Bacteriologic examination of the purulent exudate was negative. Gross and microscopic changes will be described and their possible significance discussed.

1520. EFFECT OF DESOXYCORTICOSTERONE + NaCl-INDUCED HYPERTENSION ON DIETARY ATHEROSCLEROSIS AND DIETARY HYPERLIPEMIA IN THE RAT. Quentin B. Deming,* Erwin Mosbach,* Margaret Bevans, Liese Abell,* Eugene Martin,* Lili Brun* and Evelyn Halpern.* Columbia Univ. Research Service, Goldwater Mem. Hosp., and Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.

Wistar rats were fed a Purina chow diet containing 4% cholesterol, 1% cholic acid and 0.5% thiouracil. Studies of conversion of acetate 1-C^{14} to cholesterol demonstrated that after 6 wk on this regimen there was a cessation of endogenous cholesterol synthesis. Within a period of 6-12 wk the rats developed hypercholesterolemia, hyper-β-lipoproteinemia and intimal lesions of the heart valves, coronary arteries and aorta, grossly and histologically resembling human atherosclerosis. When 2 groups of rats, one normal and one with hypertension induced by desoxycorticosterone and salt, were placed on this regimen it was demonstrated that: 1) the mean cholesterol and total lipid concentrations in the serum and total cholesterol in the livers was greater in the hypertensive group

than in the normotensive, 2) the hypertensive animals appeared to have a more severe degree of atherosclerosis than did the normals. Within the hypertensive group there was a positive correlation between the blood pressure and both the concentration of cholesterol in the serum and the estimated degree of atherosclerosis. Neither desoxycorticosterone without salt nor salt with desoxycorticosterone produced the increase in serum lipid concentrations.

1521. PATHOLOGIC STUDY OF EMBRYOS FROM VITAMIN E-DEFICIENT TURKEY HENS. T. M. Ferguson,* J. R. Couch* and R. H. Rigdon. Depts. of Poultry Science and Biochemistry and Nutrition, Texas A. and M. College System, College Station, and Univ. of Texas Med. Branch, Galveston.

Opacities in the lens of the vitamin E-deficient turkey embryos have been reported (A.M.A. Arch. Ophth. 55: 346, 1956). The most significant pathologic change present in these embryos other than in the lens occurs in the central nervous system. This lesion is characterised by focal areas of acute encephalomalacia. The most conspicuous lesions in the viscera are focal areas of necrosis in the liver. Hemorrhage and edema are frequently found in the skin and subcutaneous tissues. These changes in the brain of the turkeyembryo are similar to those described by Pappenheimer et al. (Storrs Agr. Exp. Sta. Bull. 229, 1939) in the brain of vitamin E-deficient growing chickens. These workers have studied vitamin E deficiency in the growing turkey and observed necrosis in the smooth muscle of the gizzard. We did not find any necrosis in the gizzard of the turkey embryo.

1522. METABOLISM OF TUMORS AND ITS RELATIONSHIP TO CHANGED DISTRIBUTION OF CYTOPLASMIC RIBONUCLEIC ACID DURING CARCINOGENESIS. Silvio Fiala,* Anna Fiala* and E. E. Sproul. Dept. of Pathology, Francis Delafield Hosp., College of Physicians and Surgeons, Columbia Univ., New York City.

In contrast to older data in the literature, a 100-150% increase in anaerobic lactic acid formation per cell of liver tissue of Sprague Dawley rats fed with basal diet containing 3'-Me-4-DAB developed suddenly after a lag period of 90-100 days. During this period there was already a considerable reduction in the amount per cell of respiratory elements and of respiratory enzymes such as succincxidase which persisted. The gradual slow depletion of succinoxidase and of the mitochondrial fraction parallels depletion of cytoplasmic, microsomial-bound, RNA. With the increase of fermentation, a proportional increase in the non-sedimented, macromolecular RNA takes place. Thus the metabolic transformation during carcinogenesis is linked to the changed pattern of RNA distribution in the cytoplasm. The hormones of the anterior pituitary such as FSH and ACTH provide a reverse demonstration that a relationship exists between the formation of respiratory enzymes and synthesis of microsomial-bound RNA. The level of succinoxidase does not change so long as microsomial RNA remains constant. Stimulation of the latter to further synthesis also is accompanied by a sudden increase of respiration and of succinoxidase. This suggests that interference with microsomial RNA is the fundamental feature of artificial carcinogenesis and reduction in respiration a secondary effect.

1523. SEX AND INCIPIENT ATHEROSCLEROSIS IN RATS. Louis C. Fillios* and Stephen B. Andrus. Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

Recent studies have shown that gross atherosclerosis can be readily induced in the rat (Filios et al., 1956). Furthermore, femaleness, represented either by an intact gonadal system or by replacement hormone, favors hypercholesteremia in both the rat and rabbit (Fillios, 1956; Fillios and Mann, 1956). In the present study, the role of the sex hormones on early atherogenesis was studied in 96 rats, equally represented by males, females and castrates, fed a diet containing 1.5% cholesterol and 0.5% cholic acid for 17 wk. The animals receiving hormones were injected 3 times/wk with either 1 mg progesterone, 1 mg testosterone or 0.02 mg estradiol. Our earlier findings on the influence of sex hormones on cholesteremia in the rat were confirmed. After 17 wk, females, in addition to having significantly higher cholesteremic responses, also had a greater degree of cardiovascular Sudanophilia than either males or castrates. Estradiol treatment had no effect, but testosterone significantly reduced the degree of Sudanophilia and hypercholesteremia in females. On the other hand, testosterone treatment was without effect while estradiol caused an increased hypercholesteremia and Sudanophilia among both castrates and males. Progesterone had little or no effect on the hypercholesteremic responses or the cardiovascular Sudanophilia. Generally, the degree of total Sudanophilia appeared to correlate with the serum cholesterol levels for all the experimental animals. The more severely involved animals also had early coronary intimal plaques. The relation of the above observations to the serum phospholipids, alpha and beta lipoproteins and the liver lipid components will be reported.

1524. ALTERATION OF RENAL SUCCINIC DEHYDROGENASE IN NEPHROTOXIC SERUM NEPHROSIS. Edwin R. Fisher and John Gruhn.* Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, Pa.

Fifty female Sprague-Dawley rats weighing between 150 and 200 gm were sacrificed at varying intervals up to 3 wk after receiving a single intravenous injection of 1 cc of potent anti-rat-kidney rabbit serum. The histochemical appearance and distribution of tubular alkaline and acid phosphatase, glucose 6 phosphatase, non-specific esterase, lipase and succinic dehydrogenase, as well as sulfhydryl, neutral lipid, plasmal, phospholipid, cholesterol and esters and fatty acids were compared with that observed in the kidneys of untreated controls. In addition to the well-recognized deposition of lipid in tubular epithelial cells a significant depletion of succinic dehydrogenase was observed in the proximal and distal convoluted tubules of rats receiving nephrotoxic serum. This alteration appeared as early as 18 hr following injection of nephrotoxic serum and although still apparent in animals surviving 3 wk was of less magnitude at this time and was noted only in those rats with typical glomerular lesions and proteinuria. There was no recognizable alteration in the intensity or distribution of fat-splitting enzymes examined. Correlation of this decrease of renal succinic dehydrogenase with alterations of mitochondria, the development of "hyaline droplets" and the tubular reabsorption of protein as demonstrated by the injection of T-1824 to rats with nephrotoxic nephrosis is made. The possible role of succinic dehydrogenase in regard to the latter shall be discussed.

1525. EFFECTS OF MAIZE DIETS ON RATS AND MONKEYS. Richard H. Follis, Jr. Armed Forces Inst. of Pathology, Washington, D. C.

The association of natural foodstuffs, such as corn and rice, with endemic nutritional disease in man is well recognized. Maize is deficient in certain elements

amino acids and vitamins. Exactly what role each deficiency may play in the pellagra syndrome is not clear. Since the specific effects of a lack of single essential nutrients have been delineated in various animal species, one is in a better position to study experimental syndromes produced by multiple deficiencies, particularly those which may have some bearing on endemic nutritional disease in the human. Rats of varying ages have been placed on rations of unenriched yellow corn. The following changes have been observed: disturbance in growth, loss of weight, alopecia, reproductive failure, rickets or osteomalacia with fractures of the Looser type, fibrosis of the metaphysis with large numbers of mast cells, goiter resulting from extreme hyperplasia of the thyroid epithelium, and enlargement of the pituitary due to the presence of "thyroidectomy cells". This basic maize deficiency syndrome has been modified by adding calcium, phosphorus, iodine, vitamin D, trytophan, lysine, casein and certain vitamins in various combinations. A "synthetic corn" ration has been developed; its effects on rats have been studied. In 2 adolescent monkeys (Cercopithecus griseoviridis) which have been observed on a corn diet for 30 wk, growth immediately ceased, though the weight remained fairly constant. These animals have shown no other external signs save, as might be expected, the development of clinical scurvy after 18 wk.

1526. RELATIVE ANTAGONISM OF PABA, FOLIC AND FOLINIC ACIDS TO THE EFFECTS OF SULFADIAZINE AND PYRIMETHAMINE ON TOXOPLASMA, MOUSE AND MAN. J. K. Frenkel and G. H. Hitchings,* Dept. of Pathology and Oncology, Univ. of Kansas School of Medicine, Kansas City, and Wellcome Research Labs., Tuckahoe, N. Y.

Patients treated for probable ocular toxoplasmosis with synergistic combinations of sulfadiazine and pyrimethamine (Daraprim), frequently developed moderate to severe thrombocytopenia and leucopenia which limited the dosage of drug that could be administered. However, these hematologic defects could be repaired or prevented by folinic acid (Leucovorin) administered simultaneously with the drugs. Contrary to the ready utilization of this vitamin by man, it could be shown that Toxoplasma was unable to metabolize it, indicating the usefulness of this vitamin in enhancing the therapeutic index of synergistic combinations of sulfadiazine and pyrimethamine in the treatment of toxoplasmosis. Groups of 10 mice infected with 10^5 LD_{50} of the RH strain of Toxoplasma subcutaneously were treated with the following compounds (expressed as mg% in the diet): sulfadiazine 60, pyrimethamine 30 and 100, sulfadiazine 30 plus pyrimethamine 30. These drugs were given alone, as well as with the vitamin folinic acid 6, or other chemical precursors of Co F, folic acid 60, and para-aminobenzoic acid (PABA) 100. Based on survival and cure rates, PABA was found to markedly antagonize the effect of the drugs, folic acid was moderately antagonistic, while folinic acid in smaller dosage was not significantly antagonistic. (Supported in part by Grant E-989 of the Natl. Inst. of Allergy and Infectious Diseases, NPHS.)

1527. POTENTIATION OF PSEUDOCHOLINESTERASE BY SEROTONIN AND LSD. George H. Fried* and William Antopol. Labs. of the Joseph and Helen Yeamans Levy Fndn., Beth Israel Hosp., New York City.

The antagonism of serotonin and LSD on isolated tissue responses has elicited considerable psychopharmacological interest. In attempting to extend these significant effects to the enzymatic level an investigation of the influence of serotonin and LSD on serum pseudocholinesterase activity was carried out manometircally. Fifty percent inhibition of enzyme activity was obtained with concentrations of serotonin and LSD of the order of 2×10^{-3}M and 1×10^{-6}M respectively, while marked potentiation occurred with considerably lower concentrations of these compounds. This enhancement was strikingly evident only when non-enzymatic acetylcholine hydrolysis in the presence of the drugs was subtracted from experimental values. Consideration of this control also resulted in much lower standard deviations at various concentration levels. For LSD at a concentration of 6×10^{-9}M there was a percentage augmentation of cholinesterase activity of 52±16.7, while for serotonin at 5×10^{-6}M the observed augmentation was 31±5.2 Since these lower concentrations are more likely to obtain physiologically, it is suggested that the potentiation effects may be of greater significance than the inhibition effects noted previously.

1528. COMPARATIVE EFFECTS OF COLCHICINE, NITROGEN MUSTARD AND URETHANE ON TUMOR CELLS AND BACTERIA. Nathan B. Friedman, Robert E. Hoyt* and Eileen Drutz.* Div. of Labs., Cedars of Lebanon Hosp., and Dept. of Pathology, Univ. of Southern California, Los Angeles.

There are similarities in the mechanisms by which certain agents produce neoplastic giant cells and bacterial giants. A dichotomy apparently exists between the cytoplasmic activity which leads to the synthesis of those cellular products which characterize differentiated cells and the nuclear activity prerequisite to mitosis. A comparable dissociation can be obtained between the division of a microorganism and its production of pigment. An attempt is made to correlate these phenomena with DNA/RNA ratios. (Supported by PHS Research Grant C 2490.)

1529. INCUBATION OF CHOLESTEROL-21-^{14}C WITH BOVINE ADRENAL TISSUE. Frank M. Ganis, P. Kurath and Michael Radakovich (Intr. by J. J. Morton). Depts. of Surgery (Div. of Cancer Research) and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

To ascertain the mechanism by which the side chain of cholesterol is degraded in the biosynthesis of adrenocortical hormones, cholesterol-21-^{14}C (specific activity, 6×10^4 d/m/mg) was incubated with fresh bovine adrenal gland tissue (homogenate, mince, and slice) in a Krebs-Ringer phosphate buffer (0.1 M) containing added fumarate (0.1 M), glucose (0.025 M), ATP (0.01 M), DPN (0.001 M) and ACTH. The mixture was incubated for a period of 4 hr at 38°C with gentle shaking in an atmosphere of O_2. Hourly CO_2 fractions were collected in KOH and analyzed for radioactivity. After incubation, the mixture was extracted with organic solvents and the steroid fraction obtained. This residue was purified by column and paper chromatography utilizing the solvent systems of Zaffaroni. A radioautograph of a paper chromatogram showed activity to be present in the area occupied by hydrocortisone. Upon elution and analysis of this area, hydrocortisone was isolated and identified by mixed paper chromatography and by its sulfuric acid chromogen. The hydrocortisone was found to be radioactive. No radioactivity was found in the CO_2 fractions. This evidence suggests that hydrocortisone is produced in the adrenal gland, in the presence of ACTH, by a direct scission between C-20 and C-22 of the cholesterol side chain.

1530. CHEMICAL ANALYSES OF HUMAN BILE. James L. Gilleland* and Béla Halpert. VA Hosp. and Baylor Univ. College of Medicine, Houston, Tex.

Bile from patients with cholelithiasis was analyzed for sodium, potassium, CO_2, pH, total solids, and total pigments and in some samples also for calcium, The bile was obtained from surgically removed gall bladders in 8 instances, from gall bladders at necropsy in 6, and from a tube in the common bile duct following cholecystectomy from 7 patients. The hepatic bile samples were random in 5 patients, 3 samples were obtained from another, and 24 during a period of 13 days from the remaining patient. The average values on the 24 specimens of hepatic bile from 1 individual were essentially the same as the random specimens from the other patients and similar to extracellular fluid except for the CO_2 and pigment contents. In the gall bladder bile the concentration of sodium, potassium, total solids and pigment was increased while the CO_2 and pH were decreased. These observations are in line with those previously reported on the concentration of potassium and pigment in the bile from gall bladders without calculous content. The average total daily excretion, on the patient from whom bile was collected for 13 days, was calculated as 235 mEq of sodium, 7.5 mEq of potassium, 1120 mg of pigment and 21 gm total solids. These amounts account for the values obtained in gall-bladder bile and indicate resorption from the viscus of all constituents measured including potassium and pigment, the latter in amounts estimated to be 7-and 6-fold, respectively.

1531. GROWTH OF WEST NILE VIRUS IN TISSUE CULTURES. Francis B. Gordon and Wirth I. Capps.* Naval Med. Research Inst., Bethesda, Md.

These studies were mainly concerned with the accumulation of West Nile virus in cultures of HeLa cells and of monkey kidney cells after inoculation with mouse passage virus. With moderate to high titers of inoculum in HeLa cultures the following observations were made: 1) the minimal absorption period that allowed maximal virus growth was 3 hr; shorter contact of inoculum and cells before washing gave poorer virus yields. 2) Increase in virus level of culture fluid was first detected between the 12th and 14th hr of incubation. 3) A peak of 10^5 - 10^6 mouse LD_{50}/0.03 ml was reached on the 2nd day after which the titer declined but persisted at a lower level apparently as long as any cells remained intact. 4) Cytopathogenic effects were definite on the 3rd day and progressed to complete or almost complete cell destruction a few days later. With more dilute inocula the appearance of virus in the fluid was delayed, and both the rise and decline in virus level were more gradual. Ten serial passages of the virus in HeLa cells apparently did not change its infectivity for these cells nor its pattern of growth in them. The growth of West Nile virus in monkey kidney cells gave results similar to those described above except that much less cytopathogenic effect was apparent. The cells persisted and continued to produce virus for as long as a month.

1532. TRANSPLANTATION OF MOUSE LEUKEMIAS AND LYMPHOMAS INTO GOLDEN HAMSTERS. Alfred H. Handler and Richard A. Adams (Intr. by Sidney Farber). Children's Cancer Research Fndn. and Dept. of Pathology, Harvard Med. School, Boston, Mass.

Our experience in successfully transplanting DBA/2 mouse lymphatic leukemia, P1534, into cheek pouches of golden hamsters (Fed. Proc. 15: 517, 1956) led us to study the heterotransplantability of several other strain-specific mouse lymphomas and leukemias. 6C3HED, a lymphosarcoma, and L1210, a lymphoid leukemia, like P1534, grew equally well in either cortisonized or non-conditioned hamsters. 6C3HED has been maintained for 15 passages and L1210 for 5 passages; P1534 is now in its 58th passage in non-conditioned hamsters. In contrast, C1498, originally a myeloid leukemia, has been transplantable only in cortisonized hamsters, where it has been maintained for 14 passages. All of these tumors grew rapidly in the hamster, filling the pouch by 7 days. The incidence of successful transplantation varied from 33-100%. After 5 passages in non-conditioned hamsters, 6C3HED was transplanted to mice where it grew progressively to death in C3H mice, the strain of origin, only temporarily in DBA/2 and not at all in LAF_1 and CAF_1 mice. Specificity tests for C1498 and L1210 are in progress. Metastatic lesions have been identified histologically for C1498 after the 3rd passage in conditioned hamsters. It is noteworthy that the cell line, Osgood 111, derived from a leukemia of human origin, has been successfully transplanted from tissue culture to the hamster in this laboratory, where it has been maintained for 12 passages without the aid of cortisone. (Supported in part by research grant C-2230, Natl. Insts. of Health, PHS.)

1533. PATHOGENESIS OF TRANSFERRED CHLOROLEUKEMIA IN THE ALBINO RAT. Charles Harris, William T. Burke,* Margot Gruenstein,* and Harry Shay.* Fels Research Inst. Temple Univ. Med. School, Philadelphia, Pa.

One hundred and ten suckling rats 7 days of age or less were inoculated i.p. with 0.2 cc of blood from animals with chloroleukemia. Recipient animals were sacrificed at 4-day intervals after inoculation until the 36th day and complete autopsies performed as well as studies of the peripheral blood and bone marrow. All tissues were stained with the McJunkin technique for peroxidase, and benzidine techniques were applied to blood and bone marrow smears. After 2 days no vestiges of inoculum remained in the peritoneal cavity. No evidence of transferred leukemia was found in any animal until 12 days of age. The first sign of positivity was a microscopic focus of leukemic cells in the perirenal fat. The next stage of successful transfer was manifested by green discoloration of the perirenal fat, indicating an increase of verdoperoxidase concentration in that region. Following gross spread through the connective tissues the bone marrow became affected, and only after this could the diagnosis of leukemia be made from the peripheral blood. Benzidine positivity of the cells of early microscopic lesions was less than that of the transferred cells, or of the cells in older lesions. Leukemic cells in the peripheral blood of recipient animals is a late manifestation of transferred chloroleukemia. This experiment indicates that the transfer of chloroleukemia in the albino rat is dependent upon the colonization of transplanted cells adjacent to the site of transfer, and is not the result of leukemogenic stimulation of cells of the recipient animal by a transferred particle.

1534. NEUROLOGICAL AND CYTOLOGICAL CHANGES AFTER BIS-BETA-AMINOPROPIONITRILE INJECTION. H. A. Hartmann and H. F. Stich (intr. by J. J. Lalich). Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.

Bis-Beta-aminopropionitrile (Bis BAPN) in a variety of animals will produce both psychopathologic symptoms and neurocytological changes. Injection of 1-2 g/kg Bis BAPN was effective in rats, mice, birds and fish. After a delay of 2-10 days a marked motor

hyperactivity was noted. More complex behavioral patterns, such as eating and courtship, were accentuated in the birds. In addition to an excitation of the normal behavior, Bis BAPN also produced new and abnormal patterns, e.g. backward walking (rat, mice, bird), head twitching (rat, mice) and gyroscopic movements (fish). There is a striking similarity between the responses of these animals to lysergic acid diethylamide (LSD-25) and Bis BAPN which indicates that Bis BAPN is psychopathogenic. However, LSD-25 acts immediately and has a transitory effect, whereas Bis BAPN acts after a delay of 2-10 days and apparently induces a permanent alteration of the behavioral pattern. Extensive cytological changes were evident in nerve cells of the brain and spinal cord after injection of Bis BAPN. Initially there was an increase of Nissl substance which was followed by a marked decrease after 5-6 days. Measurements of nerve cells from the anterior motor horn and from the intermediolateral column of the spinal cord showed 1/4 reduction of the cell volume, 2/3 reduction of the nuclear volume and no significant alteration of the nucleolar volume.

1535. PERIPORTAL FATTY CYSTS AND RETICULIN CONDENSATION IN LIVERS OF RATS SUBJECTED TO CYCLIC PROTEIN-FEEDING. W. Stanley Hartroft. Dept. of Pathology, Washington School of Medicine, St. Louis, Mo.

Best, Ridout and Lucas with the author previously reported (1955) the dramatic appearance of a transient fatty (periportal) liver in rats transferred on to an "adequate" diet (containing 18% casein and 0.5 % choline chloride) after a 3-wk period on a similar ration containing however only 3% casein with a supplement of 0.45% DL-methionine. In the experiments reported here, these findings have been extended. After preliminary trials, it was found that the transient nature of this type of periportal fatty liver could be overcome and the abnormal fat retained for longer periods by cyclic feeding of these rations. Forty male rats (initially weighing 120 gm) of the Wistar strain were fed the 3% casein diet for 8 days, followed by the 18% casein diet for 3 days. This cycle was repeated 10 times (total of 110 days). Forty control rats were offered and consumed equivalent amounts of a commercial stock ration. All animals gained weight on these regimens (up to 300 gm). Gross and microscopic examinations of the control animals' tissues failed to reveal abnormalities. But the livers of the rats subjected to cyclic protein feeding as described contained large fatty cysts and condensed reticulin in periportal positions. A moderate degree of pancreatic acinar atrophy was also present in the experimental rats. These findings may bear some relation to the problem of Kwashiorkor. (Assisted by grants from the Lipotropic Research Fund and the PHS grant H-1968.)

1536. INFLUENCE OF DIETARY MAGNESIUM ON CARDIAC AND RENAL LESIONS OF WEANLING RATS FED AN ATHEROGENIC DIET. Earl E. Hellerstein,* J. J. Vitale* and N. Zamcheck, Mallory Inst. of Pathology, Boston City Hosp., Depts. of Pathology, Harvard and Boston Univ. Med. Schools, and Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

Because of the possible relationships between the metabolism of 1) magnesium and thyroxine and 2) thyroxine and cholesterol, the effect of varying the magnesium in an atherogenic diet (cholesterolcholate) fed to weanling male rats was studied. The magnesium was varied from 12-192 mg/100 gm in diet containing 10% protein, 20% fat, 1% cholesterol and 0.3 % cholic acid. The animals showed gross aortic and mitral valvular sudanophilia when sacrificed at 24 days. Valvular sudanophilia decreased with increasing magnesium. Despite high dietary magnesium, the blood magnesium and growth rate (weight) were below controls (see Vitale et al., Fed. Proc., this issue). The associated renal lesion consisted of intratubular calcium deposition to which there was a response by giant cells as well as by granulocytes, predominantly eosinophils. The lesion was most marked at the outer zona intermedia, but involved the remainder of the zona intermedia and the cortex. Neutrophils and eosinophils infiltrated the peripelvic fat. The livers all showed a marked deposition of neutral fat and birefringent crystals, some of which were in a Maltese cross pattern. Animals fed the atherogenic diet but with less fat (5%) and more protein (20%) showed far less severe lesions. It is interesting that morphologically similar renal lesions are produced in rats by 1) an atherogenic diet, despite high dietary magnesium, and 2) magnesium deficiency alone.

1537. PRESENCE OF HYDROGEN PEROXIDE IN UTERINE TISSUE OF THE RAT. John B. Henry* and Fred V. Lucas. Dept. of Pathology, Columbia Univ., New York City.

It has been shown that uterine tissue of the rat contains peroxidase, but insignificant amounts of catalase and cytochrome oxidase (Lucas, et al. J. Biol. Chem. 214:775, 1955). It was also found that dilute homogenates of such tissue, when supplemented with hydrogen peroxide, will oxidize a leuco dye (2,6-dichlorobenzenoneindo-3'-chlorophenol). It is now shown that concentrated homogenates will oxidize the dye without artificial addition of hydrogen peroxide. We are thus led to conclude that uterine tissue does normally contain small amounts of hydrogen peroxide, as well as peroxidase. This view that hydrogen peroxide is present is supported by the fact that pretreatment of homogenates with catalase destroys the capacity to oxidize the dye, presumably through the removal of hydrogen peroxide. It was pointed out in the paper already cited that uterine tissue, a month after oophorectomy, contains little or no peroxidase, unless the animal is treated with estrogen. Indeed, an animal so treated, becomes a standard test animal, independent of fluctuations caused by the estrus cycle. If the uterine homogenates of such a test animal are incubated for 10 min with 17β-estradiol, the amount of hydrogen peroxide appears to have increased rather markedly.

1538. SULFHYDRYL GROUPS, ZINC, CHELATING INHIBITORS AND THEIR RELATIONSHIP TO THE ACTION OF YEAST ALCOHOL DEHYDROGENASE. Frederic L. Hoch and Bessie Zotos (intr. by James P. O'Hare). Biophysics Research Lab., Dept. of Medicine, Harvard Med. School, Boston, Mass.

The alcohol dehydrogenase of baker's yeast (YADH) has been shown to be a zinc metalloenzyme. A molecular of DPN and/or DPNH is bound to HADH at or near each of the 4 zinc atoms in the enzyme. Yeast ADH also contains sulfhydryl groups, and inhibition studies with specific reagents for -SH have been interpreted to indicate they are enzymatically active sites; no direct correlation between the analytical and the functional data is at hand. The significance of these findings for the explanation of the molecular basis of YADH action requires further study. YADH has been titrated with Ag^+ alone and in the presence of metal binding agents. The number of -SH groups measured per molecule of enzyme is a critical function of the physical-chemical parameters of the system. The presence of a variety of chelating agents, however, does not change the number of titrable -SH groups at concentrations which

inhibit enzyme activity. Similarly, the presence of DPN in a 10^5 molar excess does not alter titrable sulfhydryl content of the enzyme significantly. It may be concluded that the observed inhibition of YADH with metal binding agents is not due to their effects on -SH groups. The implications of these findings will be discussed.

1539. LIVER REGENERATION IN EXPERIMENTAL CIRRHOSIS. F. W. Hoffbauer, Cyrus P. Barnum* and F. G. Zaki. Depts. of Medicine and Physiological Chemistry, Univ. of Minnesota Med. School, Minneapolis.

Four stages in the development of dietary fatty cirrhosis in rats can be recognized: 1) centrolobular fat; 2) entire lobule fatty; 3) uniform periportal regeneration (atrophic nodular cirrhosis). The pattern of the fibrous trabeculae is determined by forces exerted by areas of regeneration. In the present study, the magnitude of this regeneration has been estimated by performing mitotic counts, and by measuring P^{32} incorporation into DNA. Seventy-one rats (Holtzerman strain, 30 days old, B. W. 100±10 gm.) were placed on a choline-deficient (8% casein) diet. Sixteen similar controls received a choline supplement (0.5%). At 10 intervals between 1 and 22 weeks, groups of animals were studied. P^{32} was injected 3 hr before sacrifice; one half of a group was studied at 7:00 a.m., and the balance at 7:00 p.m. to detect any evidence of diurnal fluctuation in regenerative activity. After 1 wk of choline deficiency, 8 rats (already at an early phase of stage I) revealed the rate of DNA synthesis and the mitotic activity to be 5-10 times the control levels. At stage 3 (3-15 wk), 37 rats showed values 7-20 times that of the control. At stage 4 (11-22 weeks), 26 rats had values 4-20 times higher than the controls. This continued high regenerative activity leads to an accumulation of liver DNA. The value expressed as DNA/100 gm body weight was more than twice as high in the cirrhotic livers as in the controls by 16 and 22 wk.

1540. NATURAL HISTORY OF ATHEROSCLEROSIS. Russell L. Holman, Henry C. McGill, Jr., Jack P. Strong,* Oscar R. Griffin,* and Jack C. Geer.* Depts. of Pathology, Louisiana State Univ. School of Medicine, and Charity Hosp. of Louisiana, New Orleans.

The usual sequence of events in human atherosclerosis—fatty streak, fibrous plaque, complication, disease—has not yet been produced in experimental animals, and the applicability of those experimental vascular lesions which have been produced to humans is not clearly established. Concern over this lack of correlation between experimental animal and man prompted us to restudy the natural history of human atherosclerosis in the light of some of the current concepts about this process. The aorta (together with the coronary, cerebral, and pulmonary arteries in some cases) in over 1000 autopsies, age 1-40 yr, from 7 different geographic areas (New Orleans, England, Spain, Puerto Rico, Costa Rica, South Africa, and Guatemala) has been stained with Sudan IV and the percentage of the intimal surface that stained red by this technique has been estimated and recorded. Every case beyond the age of 3 yr in all parts of the world thus far examined has shown some degree of sudanophilia of the intima of the aorta, and the degree of this change has increased strikingly between the ages of 8 and 18 yr, suggesting a relationship of atherogenesis to the hormonal changes of puberty. Fibrous plaques have followed fatty streaks approximately 20 yr later. These and other data to be presented do not lend support to the current concepts of passive filtration as the major mechanism involved in atherogenesis. Supported in part by grants from the Nat. Heart Inst., PHS, (H-655; H-1908; H-2048).

1541. IMMUNOLOGICAL RELATIONSHIP BETWEEN MEASLES AND DISTEMPER VIRUSES. David T. Imagawa* and John M. Adams. School of Medicine, Univ. of California, Los Angeles, and Long Beach VA Hosp., Long Beach, Calif.

Characteristic cytopathogenesis produced by measles virus (Edmonston strain) in HeLa cell cultures has been inhibited by specific canine distemper antiserum produced in ferrets. Specific distemper antiserums were prepared by immunizing ferrets with avian or mouse adapted strains of distemper virus. Following survival of a challenge dose of 100 MLD virulent ferret distemper virus, the animals were bled for serum samples. Serum from ferret 168, immunized with mouse adapted distemper virus, demonstrated neutralization of 200 TCD_{50} measles virus at dilution of 1:40. Serum samples from several ferrets, immunized with egg adapted distemper virus, showed inhibition with undiluted, 1:8, or 1:20 dilutions. On the other hand, serums from normal ferrets (including pre-immunization samples) failed to show any neutralization of measles virus. Furthermore, the effect of heated immune serum (56° C 1/2 hour) could be enhanced 2-to 4-fold by the addition of complement as present in fresh normal guinea pig serum. When inactivated normal guinea pig serum was employed, no enhancement of neutralization occurred. Ferret protection tests have been partially successful in confirming these findings. In 3 separate experiments, ferrets immunized with measles virus and subsequently challenged with virulent distemper virus, showed evidence of protection as revealed by prolonged incubation period and modified illnesses with 9 of 21 animals surviving challenge, whereas all of the 15 controls with 1 exception, died in the expected manner.

1542. PATHOLOGY OF EXPERIMENTAL MITRAL STENOSIS. William E. Jaques and Albert L. Hyman. Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans.

Thirty-three dogs were used in this experiment. An experimental mitral stenosis was surgically induced in all animals. Eight dogs also had an azygos vein ligation in order to prevent shunts into the bronchial venous system. All these animals survived more than 100 days after the development of a mitral stenosis. Cardiac catheterization was performed on many of these animals. Ascites and pedal edema appeared in 10 animals. All catheterized dogs displayed moderate pulmonary hypertension. There was generally a higher degree of pulmonary hypertension in dogs with azygos vein ligation than without. At autopsy, 17 dogs showed cardiac enlargement with definite right ventricular hypertrophy apparent in 13. The mitral valve areas varied from 1-15 mm in diameter with 8 being less than 5 mm. The livers were enlarged in 27 animals. The wall to lumen ratio and medial mass of pulmonary arterioles were determined in vessels varying from 50-150 microns. In only 3 animals was there evidence of detectable medial hypertrophy. Two of these dogs revealed a marked worm infestation. One dog showed numerous organizing thrombi with eventual eccentric intimal thickening. There was generally passive congestion of the liver and cardiac cirrhosis was present in 12 animals. Complication consisted of bacterial endocarditis in 2, dirofilarial myocarditis in 1, mycardial infarction in 1 and partial constriction of the inferior vena cava in 23 dogs. It would appear that sustained pulmonary hypertension can occur without any detectable vascular changes.

1543. COMPARISON OF I^{131} COUNTS FROM INTACT AND EXTIRPATED THYROIDS IN MICE. David L. Joftes, (intr. by Shields Warren). Cancer Research Inst., New England Deaconess Hosp., Boston, Mass.

Unanesthetized mice may be immobilized in special holding devices so that direct counting of I^{131} taken up by the thyroid may be accomplished by means of a well-collimated scintillation counter. The method was tested by recounting the thyroids which were surgically removed en bloc with trachea immediately following the counting of the thyroid in the intact animal. Extirpated thyroid recounts were begun within 5 min of the end of in vivo counts in every case. The lateral relationship between counter and tissue was preserved but the extirpated thyroids were 2-4 mm closer to the scintillation crystal than the intact glands. The 1/4" collimation aperture excluded nearly all counts originating in the salivary glands. Seventy-three Swiss albino (RAP) mice were used. Counts were performed within 2-8 hr after injection of 2 μc of I^{131}. The percentage uptakes were calculated against counts from standards prepared from aliquots of the injected I^{131} solution. The mean of the uptakes in the intact mice was 11±3.7% and the corresponding mean of the extirpated thyroids was 12±4.6%. The mean of the difference between the uptakes in the intact and extirpated thyroid was 1.7±1.6%. A large part of this difference may be explained by the difference in the distance between scintillation crystal and the gland in the intact and extirpated conditions.

1544. DEMONSTRATION OF AN ALPHA CELL MAINTENANCE FACTOR FOR RAT ANTERIOR PITUITARY IN VITRO. Irving S. Johnson and R. Barbara Mattas (intr. by Paul N. Harris). Biological Research Div., Lilly Research Labs. Indianapolis, Ind.

Roller tube cultures of anterior and posterior pituitary tissue and hypothalamic tissue from Wistar rats were made. Media was passed from hypothalamic cultures to posterior pituitary cultures and from these to anterior pituitary cultures. Media was also passed directly from hypothalamic and posterior pituitary cultures to anterior pituitary cultures while other anterior pituitary cultures were untreated. Media passage was done for 5 wk and the tissues fixed and stained by an adaptation of the Koneff modification of the Mallory-Azan stain for pituitary tissue. At the end of the passage period only anterior pituitary cultures which had received media directly from the hypothalamic cultures exhibited staining reactions of the alpha cell type. The functional significance of this finding is not yet clear, but would seem to indicate that if such a system exists in vivo, that either 1) this hypothalamic factor is not stored in the posterior pituitary and acts directly on the anterior pituitary or that 2) this factor is too unstable to resist the intermediate passage in the posterior pituitary cultures. An alpha cell of simian origin which is now nearly 2 yr old and in its 23rd serial transfer passage will also be described and possible significant differences between these rat and monkey in vitro systems will be discussed.

1545. MODIFICATION OF ARTHRITIS IN GUINEA PIG BY VARIOUS CORTICOSTEROIDS AND ACTH. Russell S. Jones and Yolande C. Mayne.* Dept. of Pathology, Univ. of Utah College of Medicine, Salt Lake City.

Acute arthritic lesions are induced in the guinea pig by a single and by 3 daily i.v. injections of a haptenic non-lethal polysaccharide complex from K. pneumoniae. The arthritic lesions appear within 6 hr after a single intravenous injection and remain up to 2 wk. Initial increase in metachromatic mucoid in joint space is soon followed by an intermingled non-metachromatic granular to globular material, then by larger masses of non-fibrinous exudate. Synovial cells at first appear vacuolated and frayed, then proliferate forming papillary projections. Later, pannus formation and periarticular fibrosis may occur. Pregnenolone, progesterone, desoxycorticosterone, corticosterone, 17 hydroxy 11 desoxycorticosterone, cortisone, ACTH and ACTH plus cortisone are injected s.c. each day in 3 mg or I.U./100 gm body weight into 200-250 gm guinea pigs. On the 7th day 1 mg/100 gm of the labeled polysaccharide complex is injected i.v., and injections of hormones continued until the animals are sacrificed 10-14 days from the onset of the experiment. ACTH, ACTH plus cortisone and cortisone reduce the synovial proliferation but augment the metachromatic mucoid and granular to globular exudate in the joint space. DOCA, corticosterone, progesterone and pregnenolone have little effect. In addition ACTH and cortisone in combination, but not alone, produce a marked ascites and unrelated small areas of necrosis in abdominal fat. Certain steroids plus the polysaccharide produce considerable bile precipitate.

1546. EFFICACY OF WATER-SOLUBLE VITAMIN K_1 IN COUNTERACTING DRUG-INDUCED HYPOPROTHROMBINEMIA. Kane L. Kelley,* Charles W. Mushett and Ralph F. Hirschmann.* Merck Inst. for Therapeutic Research, Merck Sharp and Dohme Research Labs., Rahway, N. J.

In previous studies, vitamin K_1 has been shown to possess considerably more activity than menadione or water-soluble derivatives of menadione in counteracting hypoprothrombinemia induced by drugs of the sulfa, Dicumarol or indanedione types. The current investigation deals principally with the comparative efficacies of vitamin K_1 emulsion and the disodium salt of 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate, administered intravenously, orally, or intramuscularly to dogs rendered hypoprothrombinemic by Dicumarol. Limited work was done also with the anticoagulant, dipaxin and with several other water-soluble vitamin K_1 derivatives. With both K_1 emulsion and the water-soluble diphosphate derivative, the most rapid effect followed i.v. administration. Oral treatment was less rapid and intramuscular the slowest. At equimolar dose levels, intravenously and orally, the K_1 emulsion and water-soluble material exhibited essentially similar activity. When given intravenously, the water-soluble diphosphate often appeared to act sooner than the emulsion, but within a few hours the 2 preparations showed comparable effects. Water-soluble vitamin K_1 was found to be appreciably more effective than K_1 emulsion by the intramuscular route. Data obtained in prophylactic and therapeutic studies indicate that water-soluble vitamin K_1 has a shorter duration of action than vitamin K_1 emulsion. Because of this property, water-soluble vitamin K_1 should be capable of rapidly reversing excessive hypoprothrombinemia with less likelihood of inducing refractoriness to re-institution of anticoagulant therapy.

1547. SPLENIC CHANGES IN EXPERIMENTAL CIRRHOSIS. Geoffrey Kent,* Clara Bruce,* Alvin Dubin,* Jesus de la Huerga and Hans Popper. Hektoen Inst. of Cook County Hosp., Chicago, Ill.

In ethionine-induced cirrhosis the spleen enlarges up to 10 times the norm. The enlargement is caused only to a small part by venous engorgement and

peritrabecular fibrosis. These changes, considered sequences of portal stasis, correlate with the degree of nodular reconstruction of the liver and are not influenced by discontinuation of ethionine when, despite persistence of the cirrhosis, the spleen is markedly reduced in size. The splenomegaly is caused mainly by proliferation of intermediate and large "reticuloendothelial" cells mixed with small mononuclear cells, initially in the peritrabecular zones and subsequently in clusters throughout the pulp cords. The pulp cell hyperplasia is accompanied by follicular atrophy and correlates with the degree of hepatocellular degeneration and interstitial cell reaction. It is conspicuous before any changes attributable to portal hypertension develop and disappears together with hepatocellular changes upon discontinuation of ethionine. It parallels an elevation of the serum gamma globulin and is associated with conspicuous cytoplasmic pyroninophilia removed by ribonuclease. Splenic pyroninophilia and hypergammaglobulinemia disappear upon discontinuation of ethionine. The bone marrow shows no plasmacytosis and the cytoplasmic pyroninophilia of the hepatic RE cells correlates poorly with the hypergammaglobulinemia. Against the spleen being the predominant site of excess gamma globulin formation, speaks the equally high hypergammaglobulinemia in splenectomized rats with ethionine cirrhosis. In such animals the pyroninophilia of the lymph nodes usually conspicuous in cirrhotic rats is markedly exaggerated suggesting their participation in the formation of the excess gamma globulin. (Supported by PHS Grant No. C-2030(C3).)

1548. REGENERATING BONE MARROW IN SITU: II, THROMBOPOIESIS (MOTION PICTURE). R. Kinosita, S. Ohno, and H. R. Bierman. Dept. of Cytology and Genetics, City of Hope Med. Research Inst., Duarte, Calif.

Biocinematomicroscopy of the rabbit bone marrow was carried out by a modification of a previously-reported method. This was supplemented by observations of supravital and fixed stained preparations. Many unique features in the development of the megakaryocyte were observed. The megakaryoblast develops into a polyploid megakaryocyte by repeated chromosomal reduplications without any cytoplasmic division. Hyaloplasmic, dendritic and blister-like processes may be seen to project from promegakaryocytes and the megakaryocytes, observed in supravital preparations. The promegakaryocyte, observed in situ, may actively project the dendrites. They seldom become loose and usually were drawn in as the cell maturation progresses. The granuloplasm of the mature megakaryocyte disintegrates to form granulated bodies varying in size. Mature megakaryocytes are usually located on the sinusoid walls exposed to the passage through which erythrocytes are released into the sinusoids. The granulated bodies are liberated in this intercellular space and are released into circulation together with erythrocytes. The above studies appear to present decisive contributions to some controversial issues concerning thrombopoiesis.

1549. NUCLEIC ACIDS AND PROTEINS IN THE DEVELOPMENT OF MUSCLES. Amara Kitiyakara (intr. by D. M.Angevine). Dept. of Pathology, Univ. of Wisconsin, Madison.

Leg muscles of chick embryos were studied cytochemically and chemically in order to determine the relationship between nucleic acids and the synthesis of specific muscular proteins. Proteins from dissected muscles were further separated into the following fractions, actomyosin-myosin, soluble sarcoplasm and stroma. There was a 5-fold decrease in ribonucleic acid content between the 10th and the 12th days of incubation as determined chemically. Within this period, differentiation from primitive muscle cells to bundles of well-formed fibers was observed. Localization of ribonucleic acid was found to be mainly in the surrounding mesenchyme and the periphery of the young fibers. The number of mitochondria was found to increase rapidly during the process of differentiation. Cortisone, a hormone which disturbs protein and carbohydrate metabolism, was injected into yolk sacs of chick embryos on the 6th day of incubation to determine the effect of abnormal metabolism on the formation of muscle fibers. The muscle mass of cortisone treated embryos was found to be considerably smaller than that of the controls with a relatively severe deficiency of the sarcoplasmic proteins. Muscle fibers were fragmented and bundle formation showed various degrees of abnormality. The pathogenesis of the lesions will be discussed.

1550. EFFECTS OF ETHIONINE AND HIGH FAT DIETS, WITH AND WITHOUT CHOLINE SUPPLEMENTATION ON THE PRODUCTION OF AORTIC SCLEROSIS IN RATS. Jerome Kleinerman, J. V. Klavins,* N. Kobayashi* and R. Marsters,* Dept. of Pathology, Western Reserve Univ. School of Medicine at City Hosp. Cleveland, Ohio.

Male rats of the Sprague-Dawley strain weighing 100 gm were maintained on synthetic diets for periods of 4-1/2-5-1/2 months. One group was maintained on a control diet composed of 18% casein, 11% lard, 67% glucose, 4% salt mixture and vitamins; a second group received the control diet supplemented with 0.5% choline chloride. A high fat diet composed of 18% casein, 40% lard and 38% glucose with salts and vitamins was fed to a 3rd group; a 4th group received the high fat diet with 0.5% choline supplement. A 5th group received the high fat diet with 0.2% ethionine, and a 6th group received the high fat diet with both ethionine (0.2%) and choline (0.5%). A 7th group was a "starvation" control group fed a high fat diet without choline or ethionine. All animals except those in the starvation group were fed ad lib. The most marked aortic sclerosis was found in the ethionine supplemented high fat group for in half of these animals the lesions were of severe degree, while in the remainder they were mild to moderate. The choline supplemented ethionine and high fat diet group showed only minimal lesions, similar to those found in high fat and choline diet animals. Choline supplementation appeared to prevent the production of the lesions. Total lipids, lipid phosphorous and total cholesterol determinations were performed in all groups; these showed no unusual variations.

1551. RELATIONSHIP OF HUMORAL STIMULATION OF ERYTHROPOIESIS TO IRON ABSORPTION. S. Krantz,* L. O. Jacobson and E. Goldwasser.* Argonne Cancer Research Hosp. and Biochemistry Dept., Univ. of Chicago, Chicago, Ill.

A simple method of measuring iron absorption has been developed. When mice are given Fe^{59} by force feeding and the whole animal is then counted in a well-type scintillation counter at several intervals, the fate of the administered isotope with regard to absorption or excretion can be determined. Iron is excreted for 24-48 hr after which time the total mouse radioactivity falls to a level value which represents absorbed iron. Blood levels of Fe^{59} in all cases paralleled total body count while determinations of radioiron in the excised gastrointestinal tract showed that very little was retained in this organ. It is generally known that treatment with phenylhydrazine or phlebotomy will

result in increased absorption of ingested iron and this has been demonstrated by the current method. We have also found that cobalt injected s.c. significantly increases iron absorption. All three of these situations have in common the stimulation of erythropoietin. Anemic plasma injections in mice have not as yet shown any effect on iron absorption. In an attempt to dissociate erythropoiesis from iron absorption, we found that x-irradiation (200r, 400r, 600r) increased iron absorption, presumably by damage to the intestinal mucosa.

1552. 5-HYDROXYTRYPTAMINE FORMATION FROM 5-HYDROXYTRYPTOPHAN BY MAST CELLS. David Lagunoff,* Kai Bor Lam,* Elizabeth Roeper* and Earl P. Benditt. Dept. of Pathology, Univ. of Chicago Clinics, Chicago, Ill.

Mast cells of the rat contain 5-hydroxytryptamine (5-HTA) (Proc. Soc. Exp. Biol. and Med. 90:303, 1955). The following evidence indicates that 5HTA can be formed by mast cells from 5-hydroxytrypotphan (5-HTP): material with 5HTA activity on the rat colon appears when rat skin is incubated anaerobically at 37°C in Krebs-Ringer-Phosphate at pH 7.2 with 4.5 x 10^{-3} M (DL) 5HTP. The capacity to form 5HTA in several tissue sites parallels mast cell population. Suspensions of peritoneal cells containing 5% mast cells in 0.1 M phosphate pH 7.2 form 5HTA under the above conditions. Subcutaneous areolar tissue forms 1.35 and peritoneal cells form 1.08 μg 5HTA/mm^{-3} mast cells present/2 hr. Following centriguation at least half the enzymatic activity is lost; a similar quantity of 5HTA initially present is also lost. Cells isolated in sucrose or 35% bovine serum albumin have only 1/4 the 5HTA-forming capacity present prior to isolation. The 5HTA-forming activity of skin is inhibited by heating at 60°C for 15 min and by 10^{-3} M semicarbozide. Pyridoxal-5-phosphate does not enhance the activity of skin but does enhance the activity of centrifuged cell suspensions. The pH optimum for 5HTA formation by rat skin is 7.2 and by peritoneal cells is 7.6. (Supported in part by grants from PHS, H-2141, Illinois Dept. of Welfare and Abbott Labs.)

1553. TOXICITY OF β-AMINOPROPIONITRILE FOR TURKEY POULTS. J. J. Lalich, B. D. Barnett* and H. R. Bird.* Depts. of Pathology and Poultry Husbandry, Univ. of Wisconsin, Madison.

Day-old Broad Breasted Bronze poults received varying levels of β-aminopropionitrile hydrochloride (BAPN-HCl) in a practical diet. This compound, the toxic agent of Lathyrus odoratus seeds, causes skeletal deformities and aortic rupture within 4-7 wk in rats when fed a 0.25% concentration in the diet. In turkey poults, however, concentrations of 0.125 and 0.25% produce leg paralysis and widespread hemorrhages in muscles and other organs in less than 2 wk. Pulmonary, pericardial or muscular hemorrhages were observed in 4 of 12 birds fed 0.6% BAPN-HCl in the diet. Concentrations of 0.03, 0.04 and 0.06% BAPN-HCl produced less paralysis of the legs, the poults lived longer and 15 of 50 died of abdominal aortic rupture. In contrast to the observations in test poults, weight gains were greater and infrequent hock deformities were observed in 23 control birds. Vacuolization and edema of the media with minimal focal fragmentation of elastic fibers was observed in 4 of 10 arteries of 10-wk-old control birds. Intimal thickening and intimal fibrous plaques are also present in aortas of control poults. In the aortas of poults fed BAPN-HCl, it was observed that medial edema and degeneration of the elastic and collagenic fibers invariably precedes the dissecting medial hemorrhage and aortic rupture. On the basis of this study we conclude that turkeys are more susceptible than rats to BAPN-HCl feeding. (Supported by PHS grant A538(C6).)

1554. IMMUNOLOGY AND CLINICAL COURSE OF EXPERIMENTAL NEPHRITIS WITH AND WITHOUT CORTISONE. Kurt Lange,* Lawrence Slobody,* Eugene J. Wenk* and Max Wachstein. Depts. of Medicine and Pediatrics, New York Med. College, New York City.

Experimental kidney damage produced by antirat-kidney rabbit sera in rats cannot be modified by cortisone given prior to, or simultaneously with, the nephrotoxic serum. An immediate immune reaction between the injected antibody and the rat kidney takes place. No antibody formation of the recipient animal is involved and cortisone, depressing antibody formation, fails to work. In contrast experimental glomerulonephritis produced in the rabbit by rabbit kidney localizing duck sera can be prevented or ameliorated by cortisone given prior to, simultaneously with or shortly after the immune serum. In contrast to the control animals, the formation of antibodies (precipitins) against duck serum, which are the cause of the disease, is depressed, proteinuria is absent or diminished, BUN does not rise and edema is absent. The depression of antibody formation and with it the disease is dependent on the dose of steroids. Complement (C') which falls sharply with the onset of this disease falls also in the rabbits which are protected by large amounts of cortisone. Control rabbits given large doses of cortisone only, but no rabbit kidney localizing duck serum, also show a fall in C' after 5-7 days. Thus the fall in C' in cortisone-treated rabbits with experimental nephritis is not specific. Histologic studies of the kidneys of the cortisone-treated animals and the control animals with duck serum nephritis show a very good correlation between clinical and morphologic findings. (Aided by PHS grant A-302.)

1555. NORMAL HUMAN TISSUES IN SPONGE-MATRIX TISSUE CULTURE: THEIR GROWTH PATTERNS AND INTERACTIONS WITH CANCER CELLS. Joseph Leighton. Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The invasion of normal tissues by tumor cells is being studied in vitro by combining established lines of cells derived from human cancer with cultures of normal human tissues. The normal tissues, explanted without the addition of cancer cells, have several basic patterns of growth. Cultures of the following tissues have been studied: endometrium, skin, salivary gland, prostate, lung, heart, synovial membrane, bone and brain. In some tissues, migration and growth of epithelial cells occurs within the explant, resulting in the formation of a continuous membrane of these cells around the cultivated fragment. In other tissues, the predominant pattern consists of the formation of new connective tissue as outgrowth into the plasma clot. In such cases of new growth branching capillaries as well as spindle-shaped cells have been seen. In instances where established "cancer" cell lines have been added to cultures of normal tissues, varied modes of interaction have been observed. These patterns of interaction appear to be related in part to the kind of growth exhibited by the normal tissue itself. Infiltrative spread of cancer cells into the normal tissue is commonly seen where connective tissue outgrowth predominates. When the host tissue is covered by a membrane of normal epithelium, invasion of the normal tissue is usually not seen. (Aided by Research Grant C-2800 from the Natl. Cancer Inst., PHS.)

1556. SUMMATION AND TETANUS IN POST-MORTEM HUMAN HEART MUSCLE. Eugene A. Lentini,* William C. Ullrick,* and Sheldon C. Sommers. Depts. of Physiology and Pathology, Boston Univ. School of Medicine, Boston, Mass.

Muscle columns (trabeculae carneae) from the left ventricles of human hearts were obtained at autopsy, 1-15 hr post mortem. The muscle columns were placed in a bath at constant temperature (37.5°C) and perfused continuously with oxygenated Ringers' solution. After a 30-min to 1-hr equilibration period, isometric contractions were induced with current from a square wave stimulator, the developed tension being transmitted to a strain gage, the output of which was amplified and recorded on an oscillograph. The muscle columns were stimulated at frequencies varying from 1-10/sec. For 13 preparations, 10 developed complete tetanus, 2 incomplete tetanus and 1 failed to tetanize. Marked summation occurred with complete tetanus in 8 of the preparations. In several of these, summation and incomplete tetanus occurred at frequencies of stimulation as low as 3/sec. The degree of summation and completeness of tetanus could not be correlated with the refractory period, contraction or relaxation time, or post-mortem period. Summation and tetanus often have been reported for cardiac muscle of laboratory animals, but usually only after drugs have shortened the refractory period, or, after cooling has prolonged the mechanical response. The data of the present experiments indicate that post-mortem human cardiac muscle will, in the majority of instances, show summation and tetanus at normal body temperature, and without the influence of drugs. (Supported in part by the Massachusetts Heart Assoc. and by Grant H-2488, Natl. Heart Institute, PHS.)

1557. KINETICS OF THE REACTION BETWEEN ZYMOSAN AND HUMAN, COW, AND PIG PROPERDIN. Myron A. Leon (intr. by S. S. Hudack). Dept. of Surgical Research, Saint Luke's Hosp., Cleveland, Ohio.

The kinetics of the reaction between zymosan and human, cow, and pig properdin (P) have been studied at 5°, 10° and 15° C. In all cases the formation of the complex capable of inactivating C'3 (PZ complex) follows an induction period, the length of which is inversely proportional to the reaction temperature and directly proportional to the serum dilution. The experiments support the hypothesis that one or more intermediate reactions must take place before the PZ complex is formed. This intermediate (s) may readily be removed by fractional absorption with zymosan. The implications of these findings on properdin titrations will be discussed.

1558. CYTOCHEMICAL EFFECTS OF AZASERINE AND TRIETHYLENE THIOPHOSPHORAMIDE (TSPA) ON THE RIBOSE NUCLEIC ACID (RNA) OF EHRLICH ASCITES TUMOR CELLS. Anton Lindner (intr. by Joseph J. Lalich). State Lab. of Hygiene and Dept. of Pathology, Univ. of Wisconsin, Madison.

In an earlier report it was noted that when Ehrlich Ascites Tumor cells were treated with azaserine, the concentration of desoxyribose nucleic acid (DNA) decreased while nuclear size increased. In these same cells, total protein increased with increasing cell size. In the present investigation, the behavior of RNA in the Ehrlich tumor cells in response to treatment with azaserine and TSPA was studied. Mice injected i.p. with Ehrlich Ascites Tumor cells were divided into 3 groups of 6 mice each. One group was injected daily for 6 days with 20 μg azaserine/mouse. A second group was injected daily with 16 μg TSPA/mouse, while the control group was injected daily with saline. At the end of the treatment period, ascitic fluid and tumor cells were withdrawn from each mouse and smears were made. These were stained with Azure B and quantitation of RNA was carried out using absorption microphotometry. It was found that when compared with control animals, the cells from treated mice contained less RNA. This was similar to that previously demonstrated for DNA in azaserine treated tumor cells. The response of RNA to either of the drugs used in this study appeared to be about the same.

1559. PATHOLOGY OF RADIOISOTOPIC ABLATION OF THE THYROID IN THE DOG. Stuart W. Lippincott, C. Lewallen* and C. Shellabarger.* Med. Dept. Brookhaven Natl. Lab., Upton, N. Y.

There has been a difference of opinion in recent years concerning the metabolic role of the thyroid in the dog. In conjunction with other studies of the thyroid concerning protein metabolism, the effects of the internal ionizing radiation of I^{131} have been investigated in mongrel dogs to determine 1) whether the thyroid in this species has an important role in the animal's metabolism, 2) the pathology of canine myxedema and 3) whether selective localization of this radioisotope might in time result in the production of a neoplasm in the thyroid. The possibility of the latter is a problem of importance in considerations of the justification of the clinical usage of radioisotopes for diagnostic purposes in man. (Reported studies of possible experimental carcinogenesis from I^{131} in the past have been largely confined to laboratory rodents.) Eight mongrel dogs were given a single dose by mouth of I^{131} ranging from 0.063-0.98 mc/kg body weight. All dogs developed myxedema. Clinically, this was characterized by decrease in food consumption, increase in body weight, lethargy, hyperkeratosis and epilation of skin, hyperglobulinemia, and hypercholesterolemia. Pathologically, atrophy of thyroid follicles resulted in virtually complete ablation of the gland in all dogs. Atrophy of skin and edema of corium developed. Cellular degeneration occurred in a large part of each anterior pituitary gland. A single dog had a carcinoma of the thyroid which probably bears no relation to the therapy since the other 7 animals did not have thyroid carcinoma.

1560. ESTABLISHMENT AND OPERATION OF A HUMAN TISSUE BANK EMPLOYING BETA-PROPIOLACTONE AS THE STERILIZING AGENT. Gerald A. LoGrippo. Dept. of Microbiology, Henry Ford Hosp., Detroit, Mich.

Human tissue from autopsy material has been successfully sterilized with beta-propiolactone and used clinically for homotransplantation. The artery bank has been in operation since October 1953, the bone and cartilage bank since July 1955, and the skin bank since September 1956. In addition to the bactericidal, sporicidical, fungicidal and virucidal properties of beta-propiolactone, experimental data on depth of tissue penetration for bacteria and virus sterilization will be reported. One percent beta-propiolactone will sterilize animal tissue artificially infected with vegetative and sporeforming bacteria and tissue taken from animals during the stage of bacteremia and viremia. The agent also sterilizes splenic tissue infected with the AK4- strain of mouse leukemia. Studies show that beta-propiolactone is capable of penetrating and sterilizing compact tissue (liver and spleen) to a depth of 3 mm and non-compact tissue (cancellous bone) to 6 mm. A simple, inexpensive and safe chemical procedure has been made available which can be used in small hospitals to supply the growing needs of surgeons for

sterilized and readily available tissue. The method of tissue procurement, preparation, sterilization and storage will be illustrated. Results on at least 200 arterial transplants and 70 bone transplants will be discussed. The clinical use of cartilage and skin has been limited, but the results to date will be included.

1561. CHANGES IN TISSUE PROTEINS IN THE COURSE OF CELLULAR DEATH. Guido Majno, Thomas E. Thompson* and Susi Bochet-Schupbach.* Depts. of Pathology and Biological Chemistry, Harvard Med. School, Boston, Mass.

The progression of necrosis was studied in 0.6-0.8 gm fragments of fresh homologous liver introduced into the peritoneal cavity of rats. The implants remained free for 12-15 hr, later became wrapped in the omentum and slowly organized. After intervals varying between 1 hr and 20 days the rats were killed, and the implants studied with respect to total weight, dry weight, histology, extractable proteins and optical density. The weight was increased by 8% after 1 hr and by 27-30% after 6 hr. There was a concomitant drop in dry weight (60% of normal from the 12th hr on). Aliquots of the tissue were ground in acetate buffer at pH 5.80 (ionic strength 0.15) and extracted for 1 hr at +3°C. The extract was filtered, centrifuged for 2 hr at 100,000 g and the protein content determined with a microbiuret method. The amount of extractable protein (referred to fresh weight) increased by 11% during the 1st hr, then decreased to 25-30% of normal. Optical density was determined on slices cut at 0.45 mm and examined under a microscope fitted with a spot photometer (red filter, Corning 2418). Necrotic liver was characterized by a marked increase in optical density. The early rise in weight of the implant and the accompanying increase of the extractable proteins are interpreted as phenomena of "cloudy swelling"; the increased optical density is consistent with the hypothesis that cellular proteins undergo denaturation with the formation of large aggregates.

1562. LIPOGENIC HYPERCOAGULABILITY OF BLOOD. Emanuel E. Mandel and Philippe Francois (intr. by W. B. Wartman). Chicago Med. School, Northwestern Univ. and Mount Sinai Hosp., Chicago, Ill.

Because of conflicting reports concerning the influence of fat ingestion upon blood coagulation, this problem was investigated by performing tests of coagulation and determining serum turbidity before and after breakfast. In 15 healthy subjects, receiving a high-fat meal, the clotting time of whole blood, as observed in 3 siliconized tubes, dropped from the fasting value (24-40 min) by 4-45% (mean 22%) 1 hr postprandially and 1-27% (14%) at 3 hr. The corresponding decrease of blood clotting time in plain tubes was 11-47% (mean 20%) and 5-40% (15%) from a fasting range of 9-17 min, while the shortening of the plasma clotting (recalcification) time amounted to 4-77% (28%) and 0-39 (27%), respectively, of the fasting range, 90-160 sec. Concomitantly, the serum optical density increased by an average of 51% at 1 hr and 130% at 3 hr. The clotting time changes were statistically highly significant, as compared with findings in 10 subjects when consuming a low-fat breakfast and in 5 who fasted during the test period. Two persons with fasting hyperlipemia showed no significant drop in clotting times after fat meals. The one-stage prothrombin time, performed both on undiluted and diluted plasma, did not change materially in any experiment. These results, as well as data obtained with i.v. fat emulsions, confirm Waldron's (Am. J. Med. 17:365, 1954) and contrast with Tulloch's (ibid. 14:674, 1953) conclusions. The phenomenon of lipogenic hypercoagulability appears well established.

1563. CALCIUM AND LABILE FACTOR ACTIVITY. F. D. Mann. Palo Alto Hosp. Research Fndn., Palo Alto, Calif.

When human blood was allowed to clot after mixing with an equal volume of 0.1 M $CaCl_2$, the serum showed greatly increased labile factor activity on testing with rabbit brain thromboplastin and aged plasma. This confirms the work of Flynn and Standley (1949) but greater changes were demonstrated, up to 30 times the activity of the original plasma. This high activity persisted for 24 hr, long after the labile factor activity of normal serum had disappeared. The same increase in labile factor activity was noted when oxalated plasma was recalcified with 1/4 volume of 0.2 M $CaCl_2$, about twice the usual amount. When the serum resulting from recalcification with increased calcium was mixed with thromboplastin and centrifuged, much of the labile factor activity was bound by the sediment of thromboplastin. This sediment had no thrombin activity. No appreciable labile factor activity was bound by the thromboplastic sediment when only the usual amount of calcium was used (1/4 volume of 0.1 M $CaCl_2$) or when the original human plasma was used without recalcification. (To demonstrate binding of labile factor to tissue thromboplastin it is necessary to use rabbit plasma or other rich source of labile factor). This unusual binding of labile factor to thromboplastin suggests that the increased labile factor activity resulting from increased calcium is due to formation of a complex with stable factor which is characteristically readily bound by thromboplastin.

1564. CIRCULATORY DYNAMICS OF THE CANINE SPINAL CORD. G. Margolis, A. T. Griffin, P. D. Kenan, G. Tindall, E. H. Laughlin and R. Wood (intr. by E. S. Rogers). Dept. of Pathology and Div. of Neurosurgery, Duke Univ. School of Medicine and Hosp., Durham, N. C.

The canine spinal cord is resistent to circulatory stasis, but vulnerable to injury by roentgen contrast agents. Evidence points to a vascular mechanism for the injury, with exposure period and postural relationships being critical factors. Accordingly, experiments were designed to obtain fundamental information on the rate of circulation through the spinal cord, compared to that of the cerebral cortex, and the effect of postural changes upon speed of flow. The observational method employed a standard dose of Na fluorescein, injected intra-arterially, as the indicator, with the pial vascular network viewed under ultraviolet light. The following variables of circulation were measured: appearance time, circulation time, arterial exposure period, regional venous exposure period, regional application period, general venous exposure period, and general application period. Significant observation included a circulation time of 1.9-3.4 sec for the cord and 0.9-2.0 sec for the brain, and a regional application period of 6.2-11.1 sec for the cord and 6.0-12.7 sec for the brain. Postural changes exerted no influence upon cord circulatory dynamics. Conclusions are that the cord has no intrinsic features of its circulation rendering it particularly susceptible to contrast medium injury, and that the postural factor is related to gravitational influences, increasing the concentration of the heavy contrast agents to a critical level. (Supported by PHS grants B 617 and B 1102.)

1565. ALTERATIONS IN THE HEMOSTATIC MECHANISM IN THE GENERALIZED SHWARTZMAN REACTION. Donald G. McKay, Sandor Shapiro* and

Arthur T. Hertig. Dept. of Pathology, Harvard Med. School, Boston, Mass.

Because the primary event histologically in the generalized Shwartzman reaction is a deposit of intracapillary thrombi, the effect of "Shwartzman-active" bacterial endotoxin on the blood clotting mechanism was tested. Two-kg rabbits were given 0.3 mg of Shear's polysaccharide or Meningococcus endotoxin at 24-hr intervals. Fibrinogen content, platelet counts, whole blood coagulation times, prothrombin times, antithrombic titres, fibrinolysis, fibrinogenolysis and white blood counts were then determined at intervals for the next 48-hr period. The first injection of endotoxin produced a slight drop in fibrinogen. Twenty-four hours later the fibrinogen had risen to twice normal values. The second injection at this time caused a 30-40% decrease in fibrinogen content in 4 hr. Twenty hours later it had again risen to twice normal values. A marked decrease in the whole blood coagulation time in silicone occurred 4 hr after both injections, but it rose to normal values 24 hr following each injection. The circulating platelets dropped from average levels of 300,000/cu mm to 150,000/cu mm after the first injection. The platelets remained at this low level and decreased to less than 100,000 after the second injection. During this time no fibrinolytic or fibrinogenolytic activity appeared. Also, there was no change in the prothrombin times or antithrombin titres. The marked decrease in circulating fibrinogen at the time when intracapillary thrombi are forming suggests that the "hyaline" thrombi of the generalized Shwartzman reaction are composed, in part, of fibrin.

1566. DIFFERENTIAL CENTRIFUGATION AND CHEMICAL MEDIATORS IN INFLAMMATION. Valy Menkin. Dept. of Exptl. Pathology, Agnes Barr Chase Fndn. for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Pa.

Studies on the localization of some of the chemical mediators in inflammation by differential centrifugation of canine homogenates of inflamed pleural tissue in 0.25 M sucrose have yielded the following results: 1) the nuclear, mitochondrial and the supernatant S_2 fractions were each tested in rabbits for ability to increase capillary permeability and to cause migration of leukocytes (i.e. for leukotaxine activity). A further identifying fact had shown that cortisone suppresses leukotaxine activity, whereas ACTH fails to do so, at least as far as capillary permeability is concerned. The homogenates of inflamed tissue, when the exudate is at pH 7.0 or above, contained leukotaxine-acting material in association primarily with the mitochondrial fraction, but somewhat less frequently in the microsomal fraction. The precise leukotaxine type activity was rarely recovered in the nuclear and S_2 fractions. When the pH of the exudate is acid in character no such uniformity was found. Either leukotadine activity was absent or else it was scattered without consistency in the various fractions. There was no clear-cut evidence of exudin activity in the homogenates of inflamed tissue. The activity of this mediator had been shown to be represeed by ACTH but not by cortisone. 2). The leukocytosis-promoting factor (LPF) was found in association with the S_2 supernatant fraction of the homogenates of inflamed tissue. Eight experiments indicated an average rise in the circulating leukocytes of 68.7% following the intravascular injection of S_2 in dogs. Neither of the other fractions yielded any rise in the level of white blood cells. 3). Differential centrifugation procedure was also utilized in whole exudates of varying pH. Leukotaxine was primarily recovered in association with the mitochondrial fraction of alkaline exudates. Exudin activity was found in association with the S_2 and the cellular-nuclear fraction of acid exudates. Pyrogenic effects seemed to be elicited by the S_2 fraction of alkaline exudate, whereas pyrexin-like activity from acid exudates were at times found distributed in several of the centrifuged fractions. (Aided by two grants from the PHS, Sigma XI, and Dr. A. Wander S.A., Berne, Switzerland.)

1567. COMBINED EFFECT OF DEXTRAN AND ANTICOAGULANTS IN THE NORMAL DOG. Sol M. Michaelson* and Joe W. Howland. Dept. of Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.

Several reports on the use of Dextran as a plasma expander have indicated that a defect in hemostasis may occur under specific situations. The experiments to be reported were designed to determine whether Dextran, when properly used in dogs receiving bishydroxycoumarin, ethyl biscoumacetate, heparin or phenindione would modify the hemostatic alterations produced by these agents. Dogs received 3 maintenance doses of the anticoagulants within 24 hr. They were infused with 6% Dextran, 1 gm/kg immediately after the 3rd dose of anticoagulant. Hematologic determinations including counts of white blood cells, platelets, determinations of hematocrit, erythrocyte sedimentation rate, prothrombin time, clotting time, bleeding time; and examination for clot retraction were made shortly before the 1st, after the 3rd dose of anticoagulant, and 1 hr after the Dextran infusion. After 24 hr on anticoagulants, a consistent decrease in hematocrit, with no evidence of hemorrhage, was noted. Platelet level, clot retraction and clotting time were not altered. Prothrombin time was increased with bishydroxycoumarin, phenindione and ethyl biscoumacetate. An increase in bleeding time was also seen after the latter. The only evidence of additivity on the part of Dextran infusion, was an additional prolongation in bleeding time in the phenindione-treated dogs and in clotting time in the heparin-treated animals. As suggested previously, the bleeding tendency which may occur with the improper use of Dextran is in the nature of impairment of vascular integrity rather than interference with the clotting mechanism.

1568. UREA AND CREATININE PRODUCTION IN ADEQUATELY HYDRATED SUBTOTALLY NEPHRECTOMIZED RATS. Ashton Morrison and Julius Gordon (intr. by S. Rogers). Dept. of Pathology, Duke Univ. School of Medicine, Durham, N.C.

Previous work (McCance and Morrison, Quart. J. Exp. Physiol. 41:365, 1956) indicated that reduction of the functioning renal tissue by subtotal (5/6) nephrectomy in rats led to an increased production and excretion of urea. However, the fluid intake had been somewhat restricted in the earlier experiments and the possibility existed that dehydration might account for the increased urea formation. To extend the observation and eliminate dehydration as a possible cause male rats weighing 175 gm had the right kidney and 2/3 of the left removed 8 wk before being placed in metabolism cages with access to water containing 5% dextrose. Sham-operated controls were similarly treated. All the animals were kept in the cages without food for 4 days and the daily urinary excretion of urea, creatinine and protein measured. The animals were sacrificed and the serum urea, creatinine and protein levels measured. The nephrectomized animals excreted about twice as much urea per 100 gm body weight as did the controls but the excretion of creatinin was the same in both groups per 100 gm body weight. There was a moderate increase in blood urea over the

period of the acute experiment in all the animals. No consistent alteration in the normally occurring proteinuria was discovered. Two aged males with spontaneous renal disease showed a similar increase in urea excretion on the same regimen. (Supported by a grant from the Durham United Fund.)

1569. PREVENTION AND REVERSAL OF CORONARY LIPID DEPOSITION IN RATS GIVEN ANTIKIDNEY SERUM, DESOXYCORTICOSTERONE AND SODIUM CHLORIDE. Richard Moy,* Marjorie A. Schroeder,* Audrey A. Moskowitz* and Robert W. Wissler. Dept. of Pathology, Univ. of Chicago, Chicago, Ill.

Acute lipid-containing lesions of the coronary arteries having many of the features of early atherosclerosis can be produced by antikidney serum (AKS) injection in the rat when it is followed by daily administration of a high fat diet, 1% NaCl in the drinking water and DCA injections. Previous work has shown that variation of any of these components may affect the incidence of lesions. In the present study preliminary experiments have shown that either a low fat-low protein ration or estrogen injections beginning prior to administration of AKS, DCA and NaCl will significantly decrease the incidence and severity of the coronary artery lesions. Further study has shown that the extent and severity of these lesions can also be reversed by instituting either a low fat-low protein ration or estrogen therapy after lesions have developed in the majority of the rats. A combination of these treatments has some additive effect. The blood pressure, serum lipids and urinary protein values have been studied in relation to therapy or prevention of lesions. In general when the lesions were reduced there was a marked depression of serum lipids and urinary proteins and a lesser reduction of blood pressure. A study of the tissues of these animals has revealed that estrogen therapy was most effective in decreasing fat-deposition in larger coronary arteries while the low protein-low fat ration particularly decreased lipid accumulation in the smaller coronary arteries. The mechanism of action of antikidney serum, diet etc. will be discussed in the light of histochemical study of the kidneys and arteries.

1570. ARTERIOSCLEROSIS IN PYRIDOXINE-DEFICIENT MONKEYS AND DOGS. Charles W. Mushett and Gladys A. Emerson.* Merck Inst. for Therapeutic Research, Rahway, N. J.

In "recovery" type studies, treatment of monkeys with pyridoxine, 2 or 10 mg/animal daily, for 7-14 months after a pyridoxine-depletion period of 8-9 months, failed to bring about complete regression of arteriosclerotic lesions. One monkey, sacrificed after only 11 days of treatment with 10 mg B_6 daily, showed a tendency toward normalization of enlarged organ size, characteristic of B_6 deficiency. Several monkeys fed a stock ration and injected with the antagonist, desoxypyridoxine, have not shown gross arteriosclerosis, such as was noted in animals on a B_6-deficient diet, but an occasional microscopic lesion has been seen. The desoxypyridoxine-treated monekys developed marked scaling dermatitis relatively early and also exhibited convulsive seizures and ataxia. The dermatitis responded to local treatment with pyridoxine ointment. Grossly observable arteriosclerosis was reported earlier in the ascending aorta and/or lower abdominal aorta of 2 dogs fed a B_6-deficient diet for 5.5 months followed by an average daily intake of 10γ B_6/kg body weight for 10 months. In the present group, arteriosclerotic plaques, principally in the iliac and femoral arteries, were observed in 7 of 9 dogs on test for 35 months. The average daily intake of pyridoxine per kg body weight and incidence of arteriosclerosis for the 3 subgroups follow: 1) 10γ/kg, 4 of 4 positive; 2) 100γ/kg, 2 of 3 positive; 3) 290γ/kg, 1 of 2 positive. Cardiac hypertrophy occurred in 2 animals receiving the lowest dose level of pyridoxine.

1571. INJECTION SITE FIBROSARCOMA PRODUCTION IN RATS BY FOOD COLORS. Arthur A. Nelson and Bernard Davidow,* Div. of Pharmacology, Food and Drug Admin., Washington, D. C.

In Fed. Proc. (12:397, 1953) Nelson and Hagan reported the production of numerous subcutaneous injection site fibrosarcomas with 3 FD&C colors, and few or none in nearly completed studies with 4, in rats injected with 20-30 mg/wk for 2 yr. The final tumor figures for the latter 4, in the order given there, are 4, 2, 2, and 0 per 18-rat group. Since then, 6 additional FD&C colors have been studied. Using 20 mg/wk in 2% aqueous solution, injection site fibrosarcomas of 2-8 cm diameter were produced among groups of 18 young Osborne-Mendel rats in 14 with FD&C Violet No. 1, and 6 with FD&C Orange No. 1. With the oil-soluble colors 10 mg/wk was so toxic that injections were stopped after 10 wk. With 1-1/2 mg/wk in 1% glycerin suspension, tumor production with FD&C Yellow No. 3, FD&C Yellow No. 4, FD&C Red No. 32, and (ended after 13 months) FD&C Orange No. 2 was respectively 1, 5 (?6), 0, and 0. The latter 2 colors caused hydrothorax and right-sided heart hypertrophy. There was a slight female preponderance in injection site tumors (totals 50 vs. 39). No effect on tumor production away from injection sites could be seen. Saline- and glycerin-injected controls had no injection site tumors. An additional group was injected intraperitoneally thrice weekly with 20 mg FD&C Green No. 2 in 2% aqueous solution for 2 years; no tumors were produced.

1572. EFFECTS OF MOUSE TISSUE EXTRACTS ON MITOSIS OF ASCITES TUMOR CELLS. Edwin Nishimura, Thomas Harwood,* Joseph Baum* and Paul Putong.* Dept. of Pathology, Northwestern Univ. Med. School, Chicago, Ill.

Earlier observations suggest that certain polysaccharides interfere with cell division by preventing mitotic gelatin. Heparin-like poly-saccharides presumably prevent gelatin of protoplasm in dividing cells by reducing the viscosity of the protoplasmic colloid and interfere with spindle formation. Aqueous extracts of subcutaneously implanted Ehrlich tumors (solid) in Strong A mice, tentatively indentified as containing polysaccharides, were found to lower the protoplasmic viscosity of Ehrlich ascites tumor cells by the centrifugation technique. Single injections of Strong A extracts were given intraperitoneally in Strong A mice bearing Ehrlich ascites tumor and samples were aspirated at appropriate intervals up to 24 hr or longer to determine viscosity values and mitotic indexes. Following a rapid fall in protoplasmic viscosity, a rise in the mitotic index was noted in the ascitic tumor. With gradual recovery to the normally rigid protoplasmic state of tumor cells, several hours after the injection of the extracts, the mitotic index returned to its initial level. A comparison between the ascending curve of the rising mitotic index and the curve of the theoretical tumor cell multiplication rate showed the two to correspond closely. The rise in the mitotic index was interpreted as resulting from an accumulation of cells which were arrested during mitosis. Comparison of weights of subcutaneously implanted tumors in Strong A and CAF_1 hybrid mice

showed the growth to be relatively delayed in the Strong A strain. Aqueous extracts of subcutaneous tumors from CAF_1 strain did not lower the protoplasmic viscosity of ascitic tumor cells when tested in vitro.

1573. RELATIONSHIP OF ANEMIA TO CARDIAC HYPERTROPHY. Tom D. Norman and Robert D. McBroom (intr. by Douglas H. Sprunt). Dept. of Pathology, Univ. of Mississippi Med. Ctr., Jackson.

Although it is generally accepted that cardiac hypertrophy occurs as a result of anemia, some question this as a mechanism of heart enlargement. Forman and Daniels (Proc. Soc. Exper. Biol. & Med. 28:479, 1931) studied the effect of nutritional anemia on the size of the heart and concluded that heart hypertrophy was associated with anemia. However, their data are not detailed and are complicated by nutritional factors. To determine if anemia does produce cardiac hypertrophy, the Holtzman rat was selected for study. Twenty-four rats weighing 140-160 gm each were divided into 2 groups: 12 animals to serve as normal controls and 12 animals to be made anemic by periodic injections of phenylhydrazine. The hemoglobin level in the treated rats was maintained at 5-7 gm/100 ml of blood. After approximately 8 wk the heart and body weights were obtained. The size of the heart was expressed as a body/heart weight ratio. The results of this experiment disclosed the mean body/heart weight ratio as 259.7 for the control animals and 239.1 for the anemic rats. When the "t" test was applied to these data, "p" equaled 0.10 when comparing the normal with the anemic animals. The comparison of the anemic and normal control animals did not show a significant difference between the heart weights of these groups.

1574. SYNTHESIS OF DNA IN RELATION TO NUCLEAR DIVISION IN A PLASMODIAL SLIME MOLD. Oddvar Nygaard,* Sophia Guttes* and H. P. Rusch. McArdle Mem. Lab., Univ. of Wisconsin Med. School, Madison.

DNA synthesis has been studied in relation to the division cycle in the multinucleate, ameboid myxomycete Physarum polycephalum. In this organism the nuclear divisions are essentially synchronous even in relatively large plasmodia (25-50 mg dry weight of material; E. Guttes). For the present studies the mold was grown on a soluble medium under aseptic conditions (Daniel and Rusch. Fed. Proc. 15:513, 1956). Orotic acid-6-C^{14} was added as a precursor for the pyrimidine bases of both types of nucleic acids, and thymine and uridine were isolated from the acid insoluble material as specific representatives of DNA and RNA. The specific activities of the pyrimidine bases, after exposure of the mold to radioactive medium at various times in the division cycle, indicate that RNA is being synthesized throughout the cycle, which lasted 16-20 hr under our conditions. In contrast DNA is synthesized during the relatively short time period from about 20 to about 70 min after nuclear division. No incorporation of C^{14} into thymine was found to occur in the remaining part of interphase, even after exposure of the mold to radioactive medium for as long as 15 hr. Quantitation of the isolated pyrimidine bases showed net synthesis of DNA at the time when C^{14} is being incorporated into thymine. The occurrence of DNA synthesis in the very early part of interphase in this organism suggests that synthesis of new DNA, although being a necessary prerequisite for nuclear duplication, cannot be regarded as a specific trigger for nuclear division.

1575. EFFECT OF DESENSITIZATION ON EXPERIMENTAL CAVITY FORMATION BY TUBERCULOUS ANTIGEN-ANTIBODY-REACTION. Y. Ogawa, Y. Yamamura, Y. Yamaguchi and K. Taka (intr. by P. Steiner). Toneyama Research Inst., Natl. Sanatorium, Japan, and City of Hope Med. Research Inst., Depts. of Cytology and Genetics, Duarte, Calif.

It has been previously reported by the author, Yamamura and others, that rabbits, sensitized by killed tuberculous bacilli plus adjuvant, formed typical tuberculous cavities at the site of challenge in the lung. A question arose whether sesensitization could alter the result. Thirty-one rabbits, as in the previous experiment, were sensitized by subcutaneously injecting with live or killed BCG in paraffin oil 2 or 3 times at 7-day intervals. Thirty days later, the following course of desensitization treatment started with 21 of them. The filtrate of sterilized BCG cultures was subcutaneously injected daily for 90 days in progressively increasing dosage. Tuberculin skin tests and precipitation reactions were done routinely. The results indicated development of strong sensitization and successful desensitization. Finally, all the animals were challenged by injecting with killed BCG paraffin oil in the lung. Thirty and 60 days later, the animals were sacrificed for pathological examinations. Productive changes were significantly pronounced in the challenged lungs of the desensitized animals, while intensive exudative as well as caseous changes were found in those of the non-desensitized animals. In the former group, 15 of 31 challenged lungs showed the tuberculomatous change without caseation and cavity formation. In the latter group, 12 of 17 challenged lungs presented cavities and 4 demonstrated large caseous lesions.

1576. USE OF NITRONEOTETRAZOLIUM IN ENHANCING THE PERMANENCE AND EFFECTING FINER HISTOCHEMICAL LOCALIZATION OF SUCCINIC DEHYDROGENASE. Bjarne Pearson. Detroit Inst. of Cancer Research and Dept. of Pathology, Wayne State Univ., Detroit, Mich.

A problem consistently encountered in using tetrazolium salts for histochemical studies of succinic dehydrogenase is the initial size and crystallinity of the resultant formazan and the continuence of crystal growth. This initial crystal size precludes the study of the finer localization of the enzyme. The continued crystal development for hours and days following the initial reaction affects the permanence of the preparation. To obtain a salt free from these objections 14 new tetrazolium salts were tested. Frozen sections of 5 microns were prepared in accordance with our previous procedure on INT in 1954. Best results were obtained with the following substrate: 5 cc 0.1M sodium succinate, 5 cc N,N-dimethylformamide, 5 cc 0.1M phosphate buffer and 10 mg tetrazolium salt. Organs of rats were studied using 13 incubation times each from 5-65 min. Remarkable results were obtained when 2,2'-diphenyl-5,5'-m-nitrophenyl-3,3'-(-4,4'-biphenylene) ditetrazolium (nitroneotetrazolium) was used. Its formazan is extremely find, blue and noncrystalline. It is unaffected by incubation. Examination of slides at 3 wk reveal no change. Slides rinsed in alcohol following incubation are good after 4 months. This removes lipids but does not affect the formazan. The outstanding features are extremely find blue, noncrystalline precipitate in sites of enzyme activity; in the base of cells in renal tubules, peripheral location in cross sections of myocardial fibers and in liver cytoplasm around portal areas. The sections can be readily magnified 600 times.

1577. ANTIHEMOPHILIC FACTOR (AHF): ITS INACTIVATION IN RELATION TO THROMBIN EVOLUTION. George D. Penick. Dept. of Pathology, Univ. of North Carolina, Chapel Hill.

It is known that normal plasma accelerates the evolution of thrombin in clotting hemophilic blood. Preliminary data were reported 2 yr ago (Fed. Proc. 14: 415, 1955), which showed that thrombin preparations in high concentration inactivate AHF under both in vitro and in vivo conditions. We have also shown that a $BaSO_4$ adsorbable factor is necessary for AHF to be inactivated during coagulation (Am. J. Physiol. 164:710, 1951). The role of thrombin in the inactivation of AHF by the clotting reaction has now been investigated more thoroughly; it appears to be essential. Data are presented which indicate that the necessary adsorbable factor is present not only in normal plasma, but also in plasmas deficient in AHF, PTC or Stuart Factor. It is lacking in normal serum, but can be demonstrated in sera of hemorrhagic states associated with retarded prothrombin conversion. Blood of dogs rendered hypoprothrombinemic by dicumarolization or exposure to chloroform contains little or none of the necessary factor. Plasma prepared from a severely dicumarolized dog is incapable of supporting AHF inactivation. Similarly, chloroform poisoning renders a dog resistant to the AHF-depressing effect of injected thromboplastin, apparently because of incomplete thrombin evolution. In vitro experiments indicate that the inactivation of plasma AHF is proportional to the amount of thrombin evolved or added as a purified reagent. This reaction is accelerated by calcium ions.

1578. CYTOPLASMIC PARTICLES AS SITES OF SPECIFIC PROTEIN SYNTHESIS. Theodore Peters, Jr. (intr. by C. V. Z. Hawn). Mary Imogene Bassett Hosp., Cooperstown, N. Y.

Serum albumin was detected by immunochemical techniques in desoxycholate extracts of cell particles of chicken liver prepared in isotonic sucrose. Approximate levels in μg serum albumin/gm liver are: supernate 300, "microsomes" 175, "mitochondria" 200, nuclei 10. All fractions lose albumin to the medium upon incubation of slices, but a net increase in total serum albumin still is seen. The serum albumin of the particles is not eluted by saline nor does it exchange with soluble I^{131}-serum albumin. Agar diffusion tests indicate immunological identity with circulating albumin. Upon incubation of slices in $HC^{14}O_3$, the serum albumin of cytoplasmic particles becomes labeled rapidly, and after 10-15 min represents 77-86% of the total labeled albumin present. Labeled serum albumin appears in the sucrose supernate and incubation medium after 20-30 min at an increasing rate, while in the particles the rate slows so that at 3 hr they contain only 13-17% of the total. "Microsomes" tend to become labeled somewhat faster than "mitochondria". Nuclear serum albumin does not show significant C^{14} incorporation. Upon incubation of labeled slices in non-labeled medium, the labeled serum albumin content of the cytoplasmic particles declines while that of the soluble fractions rises. The lag reported (J. Biol. Chem. 200:461, 1953) in appearance of C^{14} in albumin is not seen when the albumin in the cytoplasmic particles is included in the total. Hence the "intermediate" proposed in the above reference appears to be serum albumin antigenically reactive but not yet released from the cytoplasmic particles where it was formed.

1579. HEPATIC INTERSTITIAL CELL REACTION IN EXPERIMENTAL LIVER DAMAGE. Hans Popper Robert Stein,* Geoffrey Kent* and Clara Bruce.* Hektoen Inst. of Cook County Hosp., Chicago, Ill.

In rats exposed to injurious agents, e.g. ethionine, cells accumulate in the intralobular interstitial spaces, first periportally. In acute stages they are partially mesenchymal cells including leukocytes, and partially small cells with little cytoplasm, designated as oval cells by Farber. Although the cells show no distinct arrangement, upon bile duct ink injection fine channels appear between them confirming the previous assumption that they are bile ductular cells. Upon portal vein injection the sinusoids are only focally injected with resulting rapid ink transmission into hepatic veins; the sinusoids are compressed near severe interstitial reactions where hepatocellular degeneration usually prevails. With prolonged exposure the inflammatory cells disappear and the ductular cell nuclei elongate to resemble fibroblasts. Reticulum fibers appear, apparently in the form of basement membranes, which may eventually approximate and collagenize thus forming connective tissue septa. Near hepatocellular necroses, ductular cells may form ducts frequently with high epithelium associated with surrounding fibrosis; cholangiofibrosis. The interstitial ductular cell reaction is considered a hepatic response to injury which may accompany hepatocellular damage. It is suppressed by cortisone despite persistence of the latter and outlasts hepatocellular degeneration after discontinued injury. The unorganized appearing ductular cells are also seen in bile duct ligation with superimposed infection or granulomas of guinea-pig tuberculosis. Whether they originate from bile duct or liver cells and what their relation is to the mesenchymal cells which they resemble and with which they are frequently associated cannot yet be decided. (Supported by PHS Grant No. C-2030(C3) and Research Contract U.S. Army.)

1580. EFFECTS OF FREEZING AND STORAGE ON OXIDATIVE PHOSPHORYLATION OF MITOCHONDRIA. C. A. Privitera,* D. Greiff,* D. R. Strength,* M. Anglin* and H. Pinkerton. Depts. of Biology, Biochemistry and Pathology, St. Louis Univ., St. Louis, Mo.

A 25% suspension of mitochondria was prepared from the livers of 3-month-old, male, albino rats. 0.5 ml aliquots of the mitochondrial suspension were frozen slowly by placing them in pyrex tubes and immersing the tubes in low temperature sludges or in liquid air. Fast freezing was accomplished by spraying the suspension into pre-cooled tubes. The frozen aliquots in several tubes were thawed rapidly, pooled and the oxidation of alpha-ketoglutarate and the change in inorganic phosphate in the system measured. The remaining tubes were stored at low temperatures. Mitochondrial suspensions were frozen rapidly and slowly at -20°, -40°, -63°, -94° and -192°. At all temperatures, except -20°, activity was demonstrable after thawing. The P/O ratios of suspensions frozen slowly at -63° and either frozen slowly or frozen rapidly at -94° and -192° were not significantly different from those of the control materials which were not frozen. All activity was lost in frozen suspensions stored at -40° for 24 hr. Suspensions stored at -76° and -192° showed activity at the end of 24 hr. Maximum retention of activity was found in suspensions frozen slowly at -192° and stored at -192°. The means of the μm of phosphorus taken up (P), the μ atoms of oxygen utilized (O) and the phosphorous/oxygen ratios (P/O) were as follows: control, 17.3 (P), 6.8 (O), 2.5 (P/O); frozen slowly at -192° and thawed immediately, 13.8 (P), 6.1 (O), 2.2 (P/O); slow frozen at -192° and

stored at -192°, 14.3 (P), 6.5 (O), 2.2 (P/O). These data indicate that under carefully controlled conditions of freezing and storage the oxidative phosphorylation of mitochondria may be retained.

1581. VARIATION IN TISSUE CULTURE TECHNIQUE WITH ADAPTATION OF VACCINIA VIRUS TO EARLE'S L CELLS FOLLOWING A PROLONGED NON-INFECTIOUS PERIOD. Charles C. Randall. Dept. of Microbiology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Many strains of vaccinia virus have been cultivated without difficulty in tissue cultures of various types, W. F. Scherer (Proc. Soc. Exp. Biol. & Med. 80:598, 152) reported that vaccinia virus (Minn. Strain) caused agglutination of Earle's L strain of mouse fibroblasts but viral multiplication did not occur. Several years ago L cells and the Minn. strain of vaccinia were obtained from Scherer. The tissue cultures were maintained on glass by Earle's method and the virus propagated in chorioallantoic membranes. Conventional methods of blind passage of the agent through fresh cultures of L cells did not result in demonstrable infection, as attested by 3 separate trials (10 passages each). Late passage material was not infectious for rabbit skin or Hela cells. By contrast a modification of tissue culture method was successful in adapting the virus, as follows: L cells were inoculated in the usual manner. Following the initial cytopathogenic damage, with marked necrosis and sloughing, a few surviving cells recovered and produced a new cell population. Cultures appeared normal for several months after which plaques appeared abruptly with eventual destruction of all cells. Late random samples of cells and culture fluid stored at -45°C were not infectious for the test system, until the onset of the cytopathogenic effect. The agent obtained by the modified procedure could now be cultivated in the usual manner; regularly produced intracytoplasmic inclusions and was specifically neutralized by immune serum. Further work may relate the phenomenon to lysogeny. (Supported in part by a grant from Smith, Kline, and French.)

1582. ELECTRON MICROSCOPY OF OVALOCYTES. J. W. Rebuck, R. M. Sturrock* and R. W. Monto.* Henry Ford Hosp., Detroit, Mich.

The structure of ovalocytes was studied after formalin fixation of intact and hemolysed erythrocytes. Ovalocytes were obtained from patients with ovalocytic trait, with acquired or "pencil cells" and in one instance from a male child whose parents both possessed the ovalocytic trait. The customary oval, elliptical and bacillary forms were observed. Corpuscles from the case of elliptocytic anemia withstood the examination poorly, were deficient in hemoglobin and more elongated than the ovalocytes of the parents. In preparations shadow-cast with chromium, an unsuspected aggregation of hemoglobin at both ends of ovalocytes, elliptocytes and bacillary forms was uniformly present. Contributing to their susceptibility to increased mechanical fragility, especially in the acquired forms, was a tendency to erythroclasis. Crenation of each of the 3 forms was noteworthy in that the excrescences were formed only at the sides, not at the ends of the corpuscles. Unlike the ghosts of sickled erythrocytes, which revert to discs, the ovalocytic or elliptocytic surface ultrastructures retained their abnormal outlines after hemolysis and loss of hemoglobin.

1583. LOCAL LYMPHOID HYPERPLASIA IN THE SKIN OF THE TURKEY PRODUCED BY METHYLCHOLANTHRENE. R. H. Rigdon. University of Texas, Med. Branch, Galveston.

Recently it has been reported that the local application of methylcholanthrene to the skin of chickens resulted in a local proliferation of lymphocytes (RES Bulletin 2:#2, 40-46, 1956). These lymphocytes apparently originated from a parent cell located in or about the wall of the capillaries. The number of these lymphocytes in the chicken frequently were numerous enough to be seen macroscopically. These experiments were repeated in the turkey. A 0.25% solution of methylcholanthrene in acetone was applied locally for varying periods of time to the skin beneath the wing of young adult birds. Few small nodules approximately 1 mm in size appeared in the treated areas of skin several weeks after the methylcholanthrene was discontinued. These were composed of lymphocytes, well demarcated from the adjacent fibrous tissue stroma. Few lymphocytes were present about some of the capillaries in the dermis suggesting that they might be the beginning of one of the above described nodules. The largest lymphoid nodule observed in any turkey was 1.0 cm. It is suggested that methylcholanthrene stimulates the production of lymphocytes in the skin of the chicken and turkey in a similar manner as this carcinogen stimulates other cells in the dermis to form papillomas, fibromas, hemangiomas and neurogenic tumors in the skin of the duck (Texas Reports on Biology and Medicine 14:508-527, 1956).

1584. LACK OF INFLUENCE OF AGE OF MICE UPON THE INITIATION OF PULMONARY TUMORS WITH URETHANE. Stanfield Rogers. Dept. of Pathology, Duke Univ., Durham, N. C.

It has been reported that young mice are much more responsive to the carcinogenic effect of urethane than those mature and growing more slowly (Rogers. J. Exper. Med. 1951). One-day-old mice, however, have proved relatively resistant (Rogers. Fed. Proc. 1953; Kline. Proc. Am. Asso. Can. Res. 1954). The spontaneous occurrence of these tumors has been found related to age of the tissue (cell-family) rather than age of intact mice. (Rogers. ibid. 1955). Among animals of the same age the initiation of pulmonary adenomas with urethane has been found correlated with the growth rate of the individual at the time of exposure. It seemed feasible to compare the relative response of old and young animals to urethane without having a difference in growth rate as a variable. Upon testing, the ratio of number of tumors initiated to the number of mitoses in a standard length of ear epithelium at the time of urethane injection was found identical in old and young mouse populations. When individual old and young animals were matched as to growth rate at the time of exposure to urethane and the number of tumors compared no differences were found. These results indicate that there is no innate cellular difference in the susceptibility of old and young mice to the initiation of pulmonary adenomas with urethane and that the previously observed differences are related to growth rate at the time of exposure, young animals growing the more rapidly.

1585. "BURN TOXIN"; ABSORPTION FROM BURN SITE. Sol Roy Rosenthal and Harold Trahan.* Institution for Tuberculosis Research, Univ. of Illinois College of Medicine, Chicago.

It has been previously shown that Evans blue when injected into an air pocket underlying burned skin of the rat is absorbed into the circulation (Fed. Proc. 14: No. 405, 1955). Although previous studies indicated that injection of concentrated diffusates from burned skin ("crude toxin"—obtained by washing air pockets underneath burned skin) failed to produce

antibodies, when injected into rabbit, the use of Freund's adjuvant plus "crude toxin" resulted in relatively high titers (precipitins) in the serum of rabbit, (titers up to 1:1024). Rats burned in the usual manner contained in their serum an antigen which reacted (precipitins) with the antibodies described above. Thus it may be concluded that diffusates from the burned skin enter the blood of burned animals. Red cells from burned animals when put in contact with serum of rabbits that have been injected with "crude toxin"—adjuvant agglutinated and finally hemolyzed these cells. This was not true for normal red cells of the rat. The relationship of this finding with anemia following burns will be discussed. (Aided by a contract between the Office of Naval Research, and Univ. of Illinois, NR 114-161.)

1586. HISTOLOGICAL EFFECTS OF I^{131} ON HUMAN HYPOPHYSIS. Agnes B. Russfield, Dept. of Pathology, Massachusetts General Hosp., Boston.

Previous observations on 27 patients with thyroid disease have indicated that sparsely granulated weakly Schiff positive cells in the hypophysis, the amphophils, may increase significantly in patients with primary myxedema or carcinoma of the thyroid and appear to be suppressed by treatment with thyroid hormone. Observations on a few patients given I^{131} were inconclusive because of short periods of survival. Since publication of this work (J. Clin. Endocrinol. 15:1393-1408, 1955) hypophyses of 20 more patients have been collected, half of whom had received radioactive iodine. I^{131} given in sufficient doses to produce anatomical thyroid atrophy and clinical myxedema also appears to produce an amphophil hyperplasia in the hypophysis histologically comparable to that seen in other forms of thyroid deficiency and to accentuate the amphophil hyperplasia seen in untreated carcinoma of the thyroid. The amphophil hyperplasia produced by I^{131} can be suppressed by treatment with thyroid hormone. In some patients with carcinoma of the thyroid, tumor growth appears to be inversely related to the amphophil activity to be expected in the hypophysis. It is concluded that 1) the effects of I^{131} on the human hypophysis are due solely to the ability of this drug to produce thyroid deficiency, 2) the human amphophils are homologous to the cells of the TSH-secreting "chromophobe adenomas" which can be induced in mice by I^{131} and 3) induction of hypophyseal and/or thyroid tumors are remote but real possibilities to be considered in the use of I^{131} in man.

1587. TRANSPLANTABLE FIBROSARCOMAS OF THE PARAKEET. Hans G. Schlumberger. Dept. of Pathology, College of Medicine, Ohio State Univ., Columbus.

Fibrosarcomas arising in the retroperitoneum, no. 139; periorbital tissue, no. 320; and skin of the right wing, no. 325; of Melopsittacus undulatus have been serially transplanted in these birds. They have been carried for 3 yr through 16 passages, for 18 months through 9 passages, and for 18 months through 8 passages respectively. All are still growing vigorously. The hosts are young, genetically unrelated, and are not pretreated with cortisone or x-irradiation. Site is subcutaneous in breast. The virus etiology of transplantable fowl sarcomas suggested a similar etiology for those of the parakeet. Using tumor 139, saline extracts of glycerinated tissue from 3 different passages were each injected subcutaneously into 15 parakeets; similar extracts of fresh tumor from 4 other passages were each injected into 20 birds. In none of the 125 birds so treated was there evidence of tumor growth at the inoculation site or elsewhere. An additional 25 birds were inoculated with tumor from the 14th passage treated in vitro with 36,000 r to destroy the cells but preserve any virus present. No tumor developed at the inoculation sites. Although transplants of primary tumor 320 grew well, pieces refrigerated in 50% glycerin for 6 months did not induce neoplasia in 14 inoculated birds. Extracts of fresh second passage tumor were also non-carcinogenic for 20 injected parakeets. Extracts from the 1st and 4th passages of tumor 325, each inoculated into 20 birds, likewise failed to induce tumors. These results do not support generalizations concerning the virus etiology of avian sarcomas based solely on the fowl.

1588. EFFECT ON SERUM LIPIDS IN RATS FED PLANT OILS WITH AND WITHOUT CHOLESTEROL. Coleman R. Seskind,* Marjorie T. Schroeder,* Richard Rasmussen* and Robert W. Wissler. Dept. of Pathology, Univ. of Chicago, Chicago, Ill.

This experiment was designed to determine how the degree of saturation of dietary fat affected serum lipid levels with and without added dietary cholesterol. Six calorically balanced diets were prepared containing 50% fat, 28% carbohydrate and 5% protein. Two diets were prepared from each of 3 fats: soybean oil with an iodine number (I#) of 129, olive oil (I# 80), and 50% olive oil-50% palmitic acid (I# 40). Each diet was fed with and without 1% cholesterol. Thirty-six, 9-month, obese male albino rats were divided into 6 comparable groups. Each group was fed equal quantities of one of the diets by stomach tube 3 times daily for 10 wk. Blood was obtained biweekly and all animals were sacrificed at 10 wk. In all groups, the serum cholesterol rose to a peak at 2-4 wk and then returned to initial levels, except for the group fed 50% olive-50% palmitic plus cholesterol. Without cholesterol the different fat sources had no sustained effect on serum cholesterol levels. In groups fed cholesterol, the serum cholesterol levels increased most with 50% olive-50% palmitic, less with 100% olive oil, and with soybean there was no rise above groups fed no cholesterol. Dietary cholesterol had little effect on serum phospholipid levels so that the serum cholesterol/phospholipid ratio was elevated by cholesterol in the olive and 50% olive-50% palmitic diets but not in the soybean diet. Changes in liver and body weights will also be discussed.

1589. EFFECTS OF FAT EMULSION UPON TISSUE MAST CELLS. Harrison H. Shoulders, Jr., and H. C. Meng (intr. by John L. Shapiro). Dept. of Physiology, Vanderbilt Univ. Med. School, Nashville, Tenn.

These experiments were designed to determine if occasional reactions to i.v. fat emulsions could be explained by the liberation of histamine and related substances from tissue mast cells. These emulsions were stabilized with phosphatide and non-ionic detergents and contained no known histamine liberators. They were injected into the rat peritoneal cavity and recovered after 1 hr. The recovered fluids were assayed for histamine-like effects upon guinea pig ileum in vitro and upon bronchiolar musculature in the pithed guinea pig. Results were as follows: after recovery from the rat peritoneal cavity fat emulsions invariably caused ileal contraction and marked bronchiolar construction. The isolated ileum response could be blocked by phenergan and chemical analysis confirmed the presence of histamine in the recovered emulsions. Microscopic study of the mesentery showed disruption of mast cells following exposure to the emulsions. Control studies showed that prior

to injection into rats the emulsions had no effect upon isolated ileum and little effect upon the pithed guinea pig preparation. This was true also for fat emulsions which had been clumped by serum albumin and for unemulsified neutral fat. Studies of fluids recovered from rats injected with Tyrode's solution or 5% glucose showed no histamine activity. These studies suggest that some of the reactions to these neutral fat emulsions might be explained by the liberation of histamine and related substances. Further work indicates that the responsible agents might be the phosphatide and non-ionic components of the emulsions.

1590. CHEMICAL PATHOLOGY OF ACUTE AMINO ACID DEFICIENCIES. Herschel Sidransky* and Emmanuel Farber. Depts. of Pathology and Biochemistry, Tulane Univ. School of Medicine, New Orleans, La.

Young male and female Sprague-Dawley rats were force-fed synthetic rations devoid in a single essential amino acid. After 6 or 7 days, rats fed diets devoid of methionine, threonine or histidine were studied for certain morphologic and biochemical changes. Histologically, a fatty liver with a periportal lipid distribution was found with all 3 deficiencies. With diets devoid of threonine or histidine, large amounts of glycogen accumulated in the liver. Edema and atrophy of the pancreas and salivary glands and degenerative changes in the gastric mucosa also occurred. The force-fed control rats gained weight while the experimental rats showed little weight loss. However, in all cases the liver weights were greater in the experimental groups. Chemical analysis revealed that the increase in liver weight was largely due to lipid and glycogen. The total liver protein content was equal to or in some cases greater than the controls. Hepatic protein synthesis as measured by radioactive amino acid incorporation into liver and plasma protein and adaptive enzyme synthesis was not impaired in the experimental groups. The lipid accumulation could not be accounted for on the basis of the conversion of amino acids to fatty acids. Rats fed the same diets ad lib showed a loss of liver weight and protein without lipid accumulation. Thus, periportal lipid accumulation may be produced in the livers of rats by force-feeding diets devoid in single essential amino acids. Surprisingly, these livers do not show the decrease in protein synthesis that was anticipated.

1591. RESULTS OF INFLUENZA VIRUS INFECTION ON THE OUTCOME OF PREGNANCY IN MICE. R. A. Siem,* D. T. Imagawa,* M. H. Jones,* D. L. Chadwick* and J. M. Adams. School of Medicine, Univ. of California, Los Angeles, and Long Beach VA Hosp., Long Beach.

Influenza A virus infection (PR8) in mice inoculated intranasally early in pregnancy showed differences in the total number of pregnancies and offspring, and in the average litter size when compared with control groups. Male animals were caged with 4 females for 7 days, at which time the females were separated and divided into 6 large groups of approximately 100 animals each. Three groups received virus and the others brain heart infusion broth. Small fractions of the animals from each group were sacrificed at regular 2-day intervals for embryological and virological studies. In the 3 experimental groups, the final pregnancy rates were 36, 13 and 14%; the rates in the controls were 47, 52 and 30%, respectively. The ratio of dead to live offspring in the three experimental groups was 1:9, 1:1 and 1:6. In the controls the ratios were 1:29, 1:15 and 1:26, respectively. Litter sizes were 6.9, 5.7 and 6.7 pups in experimentals, and 6.8, 7.0 and 8.5 pups in controls. The virus inoculum was 0.3, 0.7 and 1.0 LD_{50}, respectively. Eighty to ninety percent of the experimental animals were shown to have been infected by virus as determined by individual lung assay in eggs at 72 hr post infection. Preliminary maze studies have shown no differences in learning ability of the progeny of infected and control animals.

1592. CONNECTIVE TISSUE AS A MAJOR SODIUM RESERVOIR. L. Silver,* R. W. Christie* and L. K. Dahl. Med. Dept. Brookhaven Natl. Lab., Upton, N. Y.

In 1923 Schade (Physikalische Chem., Dresden and Leipzig) proposed that connective tissues (CT) - ground substance and fibers - were importantly involved in water-electrolyte equilibria. Meyer and Rapport (Science 113:596, 1951) suggested that the mucopolysaccharides of ground substance in CT acted as cationic exchange resins. Evidence for ion-binding by these tissues is now extensive. During our studies of the relationship between sodium ingestion and development of hypertension in humans (A.M.A. Arch. Int. Med. 94: 525, 1954; Fed. Proc. 15:513, 1956) we encountered the occasional finding of rapid loss or gain in exchangeable body Na which was not explicable by external balance measurements. We concluded that some major tissue reservoir was in operation, of which CT was the most likely. To test this postulate we have studied the sodium loss of an accessible, relatively pure CT (rat tail tendon) after drastic Na depletion, following peritoneal dialysis. It has been found that significant depletion of sodium occurred, approximating 20 mEq/kg wet weight, not explicable on fall in plasma concentration. Concordant results were found in rat aorta, from which it has been deduced that CT in blood vessels may bind Na in such a manner as to affect the structure and function of the vessel. This deduction is consistent with data relating changes in Na concentration and physical properties of CT. (Mathews, Arch. Biochem. & Biophys. 43:181, 1953; Blumberg, Oster and Meyer, J. Clin. Invest., 34:1454, 1955), The fall in Na in a specialized CT like bone (Bergstrom and Wallace, ibid. 33:867, 1954) as well as other tissues (Woodbury Am. J. Physiol. 185:281, 1956) after a similar method of depletion, is compatible with the present hypothesis. It is proposed that connective tissue is an active reservoir for sodium, and such sodium, by altering the physical properties of theCT in blood vessels, plays an important role in the pathogenesis of hypertension. (Supported by the Atomic Energy Comm.)

1593. INFLUENCE OF ADRENAL RE-ENUCLEATION AND ADRENALECTOMY ON ADRENAL-REGENERATION HYPERTENSION. Floyd R. Skelton. Dept. of Pathology, Med. College of Georgia, Augusta.

Severe hypertension and vascular lesions develop during the regeneration of the adrenal cortex which occurs following unilateral adrenalectomy and contralateral adrenal enucleation in uninephrectomized rats allowed 1% sodium chloride to drink. The present experiment was performed to determine the effects of: 1) a second enucleation and 2) removal of the regenerated adrenal 6 wk after the first enucleation when hypertension was present. Results indicate that re-enucleation of the regenerated adrenal produced a transient decrease in blood pressure and saline consumption followed by a rise to above the pre-existing hypertensive levels. Complete removal of the regenerated adrenal caused a fall in blood pressure from hypertensive to normotensive levels accompanied by a drop in saline consumption to below normal limits in 19 rats, while 3 similarly treated hypertensive animals showed only a transitory fall in blood pressure

and saline intake both of which returned to pre-operative levels. Moderate to severe arteriolar lesions, especially of the kidney, were present in the 3 rats which remained hypertensive, while only slight thickening of renal arterioles was observed in 12 of the 19 rats which became normotensive. These findings have led to the suggestion that "adrenal-regeneration hypertension" is dependent initially upon some change in the functional activity of the adrenal cortex consequent to adrenal enucleation, but that it can become independent of this gland and be maintained by some other mechanism, perhaps of renal origin, somewhat like the metacorticoid hypertension which persists after cessation of desoxycorticosterone acetate administration.

1594. ENZYMATIC CHANGES IN ACUTE MYOCARDIAL ISCHEMIC INJURY. George W. Smetters,* John P. Kaltenbach* and Robert B. Jennings. Dept. of Pathology, Northwestern Univ. Med. School, Chicago, Ill.

Myocardial ischemic injury was induced in 13 dogs by permanent high ligation of the circumflex branch of the left coronary artery. Eight animals without ligation were used as controls. After periods of ischemia lasting from 40 min to 24 hr, the animals were sacrificed and samples of severely injured tissue from the posterior papillary muscle were taken for analyses of inorganic ions, glutamic oxaloacetic transaminase (GOT), succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH). Plots of the enzyme contents of the tissue against duration of injury all followed the decreasing sigmoidal curve previously described for potassium. There is an initial lag period for each component followed by a period of accelerated depletion lasting until levels of 30-50% of the enzyme content of the uninfarcted left ventricle are reached 12-15 hr after ligation. The 3 enzymes differ in the initial lag period, which is 40-70 min for GOT, 2 hr for LDH and 4-5 hr for SDH. However, once the accelerated depletion begins, the slope of the curve is essentially the same for all. These variations are perhaps due to differences in the molecular size of GOT and LDH, and to the apparent strong binding of SDH to mitochondria within the cell. The relation of these depletion curves to that previously described for potassium will be discussed. (Supported by grants from the American Heart Assoc., and the Natl. Heart Inst., PHS.)

1595. TRANSPLANTATION OF SYNOVIUM TO ANTERIOR CHAMBER OF EYE OF GUINEA PIG. Robert B. Stebbins and Russell S. Jones. Dept. of Pathology, Univ. of Utah College of Medicine, Salt Lake City.

Acute arthritic changes have been induced in the guinea pig by i.v. injection of various bacterial extracts. Localization of bacterial polysaccharide complexes has been observed in the synovium by C^{14} labeling. Visualization of the synovial changes has been attempted by transplantation of synovium from the knee joint into the anterior chamber of the same guinea pig. After 1 or 2 wk the labeled bacterial polysaccharide is injected i.v. and the animals are sacrificed at various intervals. Before sacrifice, the changes in the transplanted tissue are observed with the dissecting microscope. The well-vascularized transplants become engorged but the fluid of the anterior chamber does not become cloudy. Comparison of the changes in the synovial transplant with the synovium of the non-operated joints is difficult because of the effect upon the transplant of reactions to small areas of necrotic fat and to occasional proliferation of bone and cartilage. Autoradiographs of the transplants show localization of the labeled polysaccharide similar to that of the synovium of intact joints.

1596. ISOLATION AND CHEMICAL PROPERTIES OF FOLLICLE STIMULATING HORMONE. Sanford L. Steelman* and Albert Segaloff. Depts. of Biochemistry, Baylor Univ. College of Medicine, and Univ. of Texas M. D. Anderson Hosp., Houston; and Endocrine Research Lab., Alton Ochsner Med. Fndn., New Orleans, La.

Follicle stimulating hormone (F.S.H.) has been isolated from swine pituitary glands in apparently pure form (Endocrinology 59:256.1956). The starting material was digested with pancreatin and purified by ethanol precipitation at pH 8.6 and 4.5. However, it has been found that enzymatic digestion is not essential for the preparation of highly active F.S.H. Final fractionation was accomplished on DEAE-Cellulose using gradient elution with sodium chloride (0.25-0.5 M) and disodium phosphate (0.05-0.1 M). The preparation was homogeneous by paper electrophoresis, ultracentrifugal analysis and chromatography. The F.S.H. activity (method of Steelman and Pohley) is 30-40 times the Armour Standard (264-151X). The amino acid composition and isoelectric point are different from previously published data on "pure" sheep F.S.H. The carbohydrate fucose is present in addition to mannose, galactose and hexosamine. The approximate molecular weight is 29,000. In hypophysectomized rats ovarian follicular and testicular tubular growth are stimulated. Even 250 γ daily for 3 wk does not produce testes of a weight equal to that which can be produced with mixtures of gonadotrophins. A total dose of 500γ will double ovarian and increase uterine weight by 50% in hypophysectomized female rats.

1597. "SHOCK" FROM EXTRACTS OF HOMOLOGOUS OR AUTOLOGOUS LYMPHOID TISSUE. Herbert C. Stoerk and Tatianja Budzilovich.* Merck Inst. for Therapeutic Research, Rahway, N. J.

During a study of immunity to homologous tissue we had observed that i.v. injections into rats of extracts from rat thymus or lymphnode—but not from a variety of other organs—were followed by a state of shock associated with mydriasis, dyspnea and prolonged inactivity. Larger doses caused convulsions and death. Thymectomized rats, injected with extracts of their own thymus, exhibited the same symptoms. This phenomenon has been investigated further. Extracts of lymphoid tissue of various animal species were prepared by homogenizing 1 part of tissue with 9 parts of physiological saline. The homogenates were then centrifuged or filtered and the supernatant or filtrate injected intravenously. Two to three ml/kg of body weight of such extracts from rat thymus caused definite shock in rats but even larger quantities failed to produce any sumptoms in animals of other species (guinea pig, rabbit, cat, dog, chicken). Conversely, extracts from lymphoid tissue of species other than rat (chicken, guinea pig, rabbit, cat, calf, monkey) even in relatively large quantities proved innocuous to rats but caused severe "shock" in the animal species from which they originated. Antihistamines, atropine, adrenaline or pretreatment with large amounts of cortisone did not modify the animals' reaction to the extract. However, pretreatment with sublethal amounts of extract caused "desensitization" inasmuch as fatal doses were well tolerated by such animals.

1598. ATHEROSCLEROSIS IN MONKEYS WITH MODERATE HYPERCHOLESTEROLEMIA INDUCED BY DIETARY CHOLESTEROL. C. Bruce Taylor, L. G. Nelson-Cox,* B. J. Hall-Taylor,* and G. E. Cox.* Dept. of Pathology, Presbyterian-St. Luke's Hosp., Chicago, Ill.

In earlier studies in monkeys ingesting a nutritionally adequate diet containing 15% butter-fat and 1.5% cholesterol, we reported the rapid formation of atheromato only at sites of vascular injury, in animals with serum cholesterol levels of 250-375 mg% (Circulation. 10:603 and 613, 1954). Atheromata were not demonstrated in either injured or uninjured tissue in control animals with cholesterol levels between 124 and 184 mg%. Recently, atheromata have been demonstrated in uninjured areas in the aortas of 5 cholesterol-fed monkeys following longer periods (6-24 months) of moderate hypercholesterolemia (mean levels of serum total cholesterol ranging between 256-393 mg%). Until sacrificed, all animals remained healthy with slow progressive weight gain. Grossly, lesions consisted of small pin-point, yellow plaques or a confluence of several of these small lesions. Microscopically, lesions consisted of an accumulation of both intracellular and extracellular lipids in a thickened intima. Superimposition of arterial injury in hypercholesterolemic monkeys appears to telescope into a few weeks a degree of arterial degeneration with intimal thickening and atheromatosis which simulates that occurring in humans over a period of years. This study demonstrates that arterial injury produced by freezing is not necessary to the dietary production of vascular atheromatosis in the monkey if studies are extended over longer periods of time. (Supported by the Chicago and American Heart Assocs., Life Insurance Med. Research Fund and the Otho S. A. Sprague Memorial Inst.)

1599. EFFECT OF GLUCAGON ON PERIPHERAL UTILIZATION OF GLUCOSE. H. H. Tomizawa,* P. M. Hyde,* E. Larson* and R. H. Williams. Dept. of Medicine, Univ. of Washington School of Medicine, Seattle.

Glucagon has been reported by various investigators to enhance, inhibit, or not affect peripheral utilization of glucose. In support of the concept of enhancement, Elrick et al. (J. Clin. Investigation, 34:1830, 1955) reported that after the injection of a partially purified glucagon preparation into depancreatized dogs, there was a persistence of an increased arterio-venous glucose difference after the hyperglycemic peak. In the present investigation, similar experiments using crystalline glucagon were carried out on totally depancreatized dogs. After food and insulin were withheld for 16-18 hr, 250-300 μg of glucagon were injected intravenously. Arterial and venous samples for glucose determinations were drawn from the femoral vessels every 10 min for approximately 2 hr. Results from these experiments were compared with those in which 84-112 mg glucose/min were infused intravenously into the same dogs over a 50-to 60-min period. In both cases, there was initially an increased A-V glucose difference, which did not persist after the hyperglycemic peak. Therefore, no direct effect of glucagon on peripheral utilization of glucose was detected by this technique.

1600. GROWTH OF EMBRYONIC GUT ON EXTERIOR CHEST WALL OF CORTISONIZED HOSTS. Helene Wallace Toolan. Surgical Research Unit, Ft. Sam Houston, Tex., and Sloan-Kettering Inst. for Cancer Research, New York City

Embryonic gut can be grown readily on the chest wall of cortisone-treated adult rabbits or rats. The skin of the host animals is removed down to the panniculus and the gut is placed as minced pieces or whole sections on the prepared areas. Such grafted material matures into adult type gut with actively secreting goblet cells. The surface, which faces the exterior and grows laterally to meet and lie adjacent to the ingrowing epithelium is moist, pink and healthy; it resembles adult gut lining in every way and can be maintained in this condition as long as the host is cortisonized. The younger the donor embryo the better results are obtained in grafting.

1601. VASCULAR DISEASE PRODUCED BY COMBINATIONS OF HYPERCHOLESTEROLEMIA AND HYPERVITAMINOSIS D. Richard E. Trueheart,* Marjorie Stumpe* and George M. Hass. Lab. of Pathology, Presbyterian-St. Luke's Hosp., Chicago, Ill.

The administration of large amounts of vitamin D leads to the production of an experimental vascular disease resembling Monkeberg's sclerosis but this disease differs from the usual human disease in that it lacks an atheromatous component. It has occurred to us that an experimental vascular disease more similar to human disease might be produced by superimposing a state of hypercholesterolemia, thus adding an atheromatous component. It was found that the simultaneous or sequential production of these 2 metabolic forms of vascular disease in the same rabbit results in several patterns of athero-arteriosclerosis which were a reflection of the rate of development, sequence, severity and duration of each of the 2 fundamental disturbances: one primarily affecting the media and secondarily the intima of special arterial systems; the other primarily affecting the intima and secondarily the media of the same or other arterial systems. Serial studies of the disease process show that an important basic change included the production of an intimal stromal reaction which was secondary to the medial disease. This stromal reaction, excited by medial disease due to hypervitaminosis D, consists of fibro-elastic intimal proliferation. When a state of hypercholesterolemia is superimposed, this reaction was converted into a fibro-elastic-atheromatous proliferative reaction. These observations lead to a view that various forms of experimental disease which resemble various forms of human athero-arteriosclerosis resulted from a combination of basic changes in the arterial wall with changes in the composition of plasma lipids.

1602. RADIATION-INDUCED "DENTAL DEATH" IN MICE. A. C. Upton, R. F. Buffett,* J. Furth and D. G. Doherty.* Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn., and Children's Cancer Research Fndn., Boston, Mass.

Mice of the RF and LAF_1 strains developed dental deformities after exposure of the head to a single dose of 1250-1800 r of 250 kvp x-rays at 9-10 wk of age. Mice affected severely were unable to feed adequately on food pellets and gradually starved to death unless provided with a soft diet. One or all of the incisors, more commonly the maxillary, were affected by varying degrees of malformation, loss, erosion of the crown, or tipping, accompanied rarely by hypertrophy. Loss of teeth was attended by ulceration of the gums and abscess formation. The time of onset of dental deformities varied with the dose of radiation, all mice exposed to 1300 r exhibiting dental changes within 70 days after irradiation. Administration of 9 mg of 2-mercaptoethylguanidine·HBr (prepared from S,2-aminoethylisothiuronium·Br·HBr) intraperitoneally

10 min before irradiation afforded marked protection against these changes. Dental deformities were not observed in animals exposed to doses below 1000 r or in nonirradiated controls.

1603. HYPERTROPHY OF THE HEART IN GUINEA PIGS LIVING AT SIMULATED HIGH ALTITUDE. Enrique Valdivia (intr. by D. Murray Angevine). Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.

Generalized increase in the capillary bed has been demonstrated in guinea pigs living at simulated altitude of 18,000 ft for prolonged intervals. Van Liere and others have reported an increase in the weight of the heart of guinea pigs living at simulated high altitude and have attributed this to chronic hypoxia. Rotta and Hurtado have reported evidence of hypertrophy of the right heart in humans born and living in the Peruvian mountains. The hearts of 22 guinea pigs kept at simulated high altitude were studied to determine the site of hypertrophy. The total weight of the heart is increased. Marked hypertrophy of the right ventricle, appearing at the end of the 2nd wk and being conspicuous at the end of the 4th wk, is demonstrated in the experimental animals when compared with controls. The left ventricle also presents minimal hypertrophy, as well as both atrial walls, especially the right. It is possible that several factors may be of etiologic significance. Chronic hypoxia acting upon the myocardium does not alone explain these findings because of the inequality of the hypertrophy of the ventricles. Increase in the total blood volume that occurs would affect primarily the left ventricle. Since increased pressure or other changes in the pulmonary vascular bed may be the significant cause, special studies of this area are being made. (Supported by a grant from the Muscular Dystrophy Assoc. of America.)

1604. FATE OF THE CELL PARTICULATES IN REGENERATING RAT LIVER AS MEASURED BY REFERENCE ENZYME ACTIVITY. J. L. Van Lancker and D. Sempoux (intr. by William H. Carnes). Dept. of Pathology, Univ. of Utah, Salt Lake City, and Univ. of Louvain, Belgium.

From comparison of the data obtained in different laboratories, A. L. Greenbaum, (J. of Physiol. 125:25, 1954) and G. Weber (Cancer Research 15:679, 1955) concluded that during liver regeneration the microsomial enzyme activities returned to normal faster than the mitochondrial. In the present work the activities of cytochrome oxidase, acid phosphatase, and glucose 6-phosphates were measured 6, 12, 24, 36, 48, and 72 hr after partial hepatectomy in rats. These were compared with their activities in the normal fraction of liver obtained at operation. The enzymes were thought to be representatives of the various cell particulates, the mitochondria, the lysosomes, and the microsomes respectively. Their activities were measured in the homogenate. Total DNA and total N assays, as well as nuclear counts, were performed at the same time. The DNA and all the enzyme activities per nucleus increased rapidly after hepatectomy. Twenty-four hours after operation the DNA per nucleus reached a maximum of 200% of normal, the mitochondrial enzyme activities per nucleus were 170%, and the microsomial enzyme activity per nucleus was 155%. These 24-hr peaks were followed by a rapid return to normal. Forty-eight hours after operation the values per nucleus were only 20% above normal for the DNA and the mitochondrial enzymes and 15% above normal for the microsomial enzymes. In the normal liver the acid phosphatase is 80% particulate bound while 20% is measured as free acid phosphatase. These relative values were not changed in the source of liver regeneration.

1605. IMMUNOCHEMICAL STUDY OF LESIONS IN EXPERIMENTAL SERUM SICKNESS. Jacinto J. Vazquez,* Charles G. Cochrane* and Frank J. Dixon. Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

An attempt was made to demonstrate specific antigen and/or antibody in the renal lesions of experimental serum sickness by means of the "fluorescent technique" of Coons et al. Glomerulonephritis developed in approximately 85% of rabbits after a single i.v. injection of purified bovine serum albumin (BSA). Lesions were present in animals sacrificed 1 day before; the day of; 1 day after and to a lesser extent 3 days after complete antigen elimination. The average time of complete antigen elimination was 10 to 13 days after antigen injection. Specific, selective localization of BSA was found in altered glomeruli. This localization of antigen was demonstrable in all glomerulonephritic kidneys. Attempts to demonstrate specific antibody (anti BSA) in similar locations have been unsuccessful. As controls, kidney sections of normal rabbits; of rabbits sacrificed 4 days after antigen injection and of rabbits sacrificed on the 1st day of immune antigen elimination were studied. Another control group consisted of rabbits who received 400 r of total body irradiation prior to the antigen injection and were sacrificed 11 days later. Control tissues had neither lesions nor localization of antigen in the glomeruli indicating that antigen did not localize in glomeruli in the absence of an antibody response. A greater concentration of homologous gamma globulin was found in diseased glomeruli than in control glomeruli. It is concluded that antigen is specifically localized in the glomerular lesions of serum sickness during the antibody response and for several days after the elimination of circulating antigen.

1606. MORPHOLOGY AND HISTOCHEMISTRY OF EXPERIMENTAL NEPHRITIS IN THE RABBIT WITH AND WITHOUT CORTISONE. Max Wachstein and Kurt Lange.* Dept. of Pathology, Saint Catherine's Hosp., Brooklyn, and Dept. of Medicine, New York Med. College, New York City.

Experimental nephritis was produced by the administration of rabbit kidney localizing duck sera. Animals were sacrificed after various intervals and sections for microscopic study stained with routine and histochemical technics. Changes observed were correlated to clinical and laboratory findings. Following the administration of the serum there exists a latent period of 5-7 days in which a moderate proliferation of endothelial cells is seen in the glomerular capillaries. With the appearance of clinical disease, alterations in the glomeruli become very prominent. There is marked increase of cells in glomerular tufts, thickening and edema of capillary walls and deposition of intensely PAS positive material within capillary lumina and in Bowman capsules. The tubules contain many PAS positive casts. With the appearance of proteinuria the proximal convoluted tubules become filled with hyaline PAS positive droplets presumably reabsorbed protein. These droplets disappear after 5-6 days. With their disappearance, degenerative changes in the tubules become manifest. There is marked cytoplasmic vacuolization followed by atrophy of tubular cells and focal concomitant regenerative changes. Various histochemical reactions show no significant changes in the early phase. Six to 8 days after the onset of clinical disease, depression

of various enzymatic activities is, however, seen in the proximal convoluted tubules. Such depression is particularly noticeable in sections stained for alkaline phosphatase, glucose-6-phosphatase, succinic dehydrogenase and DPN diaphorase activity. In cortisone-treated animals a distinct correlation is observed between functionsl and anatomical changes. The degree of protection against the disease provided by cortisone is closely reflected by the microscopic findings. (Aided by grant A-688 of the PHS.)

1607. RELATIONSHIP OF THE ASCHOFF BODY TO EXPERIMENTAL MYOCARDITIS. Bernard M. Wagner. Dept. of Pathology, Children's Hosp. of Philadelphia, Philadelphia, Pa.

The evolution of the Aschoff body has been studied in left auricular appendages removed during the course of mitral commissurotomy for rheumatic mitral stenosis. A total of 512 specimens have been reviewed of which 130 have been investigated by a variety of histochemical, cytochemical and immunochemical methods. Particular attention has been directed to the earliest histological changes: collagen fiber swelling and fragmentation, accumulation of acid mucopolysaccharide and the origin of fibrinoid. Myocarditis was produced in 4 groups of young male rabbits by the i.v. administration of meningococcal toxin, horse serum, lipopolysaccharide from E. coli (Landy) and lipopolysaccharide from E. coli (Westphal). An additional group of animals was treated with x-ray (400 r) and then given toxin. A total of 60 rabbits were studied. Evidence will be presented that human rheumatic carditis is a highly specific, unique phenomenon. The pathognomonic lesion has not been observed in experimental animals. Histochemical analyses will demonstrate specific differences between the human and experimental diseases. Preliminary results utilizing fluorescein-labeled antibodies to Group A streptococcal antigens and their implications will be discussed. Electrophoretic patterns and serological data from patients with tissue evidence of active rheumatic carditis will be examined along with similar data obtained from rabbits with histological findings of myocarditis.

1608. PARENTERAL ADMINISTRATION OF ANTI-HEMOPHILIC FACTOR (AHF) PREPARATIONS IN THE DOG. R. H. Wagner,* R. D. Langdell,* B. A. Richardson,* R. A. Farrell* and K. M. Brinkhous. Dept. of Pathology, Univ. of North Carolina, Chapel Hill.

Previous studies have been made of plasma anti-hemophilic factor (AHF) levels following i.v. injections of AHF fractions in normal and hemophilic dogs (Proc. Soc. Exp. Biol. & Med. 88:212, 1955). After injections, the plasma AHF levels of the hemophilic dogs were raised to normal and then fell to low levels in a few hours. The plasma AHF of the normal dogs after injection quickly returned to preinjection levels. This rapid disappearance of i.v. injected AHF has been further studied in order to obtain better estimates of the time required for the AHF to equilibrate with the extravascular spaces and of the time required for the subsequent disappearance from the total plasma pool. In addition, the feasibility of other possible routes of administration have been studied. Plasma AHF levels were measured in hemophilic dogs following i.m. and s.c. injections of varying amounts of AHF fractions. AHF promptly appeared in the plasma after either type of administration, but higher plasma AHF levels were reached with i.m. injections than with the same amount of AHF given s.c. The duration of assayable AHF levels in the plasma after i.m. injection approached that observed after i.v. administration of the same dose.

1609. SEROLOGIC STUDY OF ANTI-LEUKOCYTE ANTIBODIES IN MAN AND IMMUNIZED RABBITS. Roy L. Walford. Dept. of Pathology, Univ. of California School of Medicine, Los Angeles.

The sera of 3 patients giving a strongly positive leukoagglutinin test and the sera of rabbits immunized with lyophilized human white blood cells were used to investigate leukoagglutinins, leukoprecipitins, agglutination of antigen-coated tanned sheep erythrocytes, and the effect of anti-leukocyte antibodies upon leukocyte amoeboid motility. The patients had hematologic diseases and had received multiple transfusions. Their sera demonstrated leukoagglutinins in a titer of 1-4 to 1-16; and showed significant inhibitory effect upon amoeboid motility after prolonged (18 hr) incubation with viable white blood cells, as judged by phase-contrast microscopy. No inhibitory effect could be demonstrated with 1 or 4 hr of incubation. Control sera showed no significant inhibitory action upon leukocyte amoeboid motility after either 4 or 18 hr of incubation. Leukoprecipitins and antibodies for antigen-coated tanned sheep erythrocytes were absent or not demonstrated in the human sera, but were present in immune rabbit sera. By precipitation in an agar medium, 2 separate antigens were demonstrated in lyophilized white cell mass by use of immune rabbit sera. The role of anti-leukocyte antibodies in the manifestations of certain hematologic diseases has received emphasis in recent literature. Of equal interest is the possibility that the development of homospecific anti-leukocyte antibodies may represent a variety of the so-called homograft reaction, one that is amenable to in vitro serologic study. A delayed cytotoxic effect, manifested in this instance in the amoeboid motility studies, might be of basic importance in the homograft reaction.

1610. ELECTROPHORETIC STUDIES ON SERUM PROTEINS OF MICE, GUINEA PIGS AND RABBITS WITH AUTOLOGOUS OR HOMOLOGOUS SKIN GRAFTS. Alvar A. Werder, Creighton A. Hardin* and Perry Morgan.* Depts. of Med. Microbiology and Surgery, Univ. of Kansas School of Medicine, Kansas City.

Measurements were made utilizing paper electrophoresis (Spinco Model R) on the relative concentrations of serum proteins in 560 mice, 56 guinea pigs and 37 rabbits previously transplanted with autologous or homologous skin. Preliminary to these experiments the following determinations were obtained: 1) the mean values for protein concentrations of normal animals in each species, 2) the effect of using pooled serum and 3) the effect of freezing and thawing serum from 1-5 times. The mean values of the relative concentrations (in %) of the serum proteins in the control animals were as follows:

	Albumin	Alpha Globulin	Beta Globulin	Gamma Globulin
Mice	42.1	13.6	29.3	14.9
Guinea pigs	49.0	26.2	15.0	9.8
Rabbits	59.1	15.9	16.5	8.4

The concentration of the serum proteins were not altered significantly from the above mean values when 1) using pooled samples or 2) freezing and thawing serums. No marked differences were found when comparing the concentrations of serum protein in the animals grafted with 1) autologous and 2) homologous

skin grafts. However, there were species differences in the resulting electrophoretic patterns. In mice alpha globulin was increased and beta globulin lowered. In guinea pigs and rabbits albumin was lowered and gamma globulin increased. It appears that the results are due to stress, injury or healing mechanisms.

1611. RIGHT-SIDED THROMBOTIC LESIONS PRODUCED IN NORMAL DOG HEART BY PERIPHERAL VENOUS EMBOLI. Stanford Wessler,* David G. Freiman and Jay H. Katz.* Harvard Med. School and Beth Israel Hosp., Boston, Mass.

The systemic infusion of canine serum induces, in dogs, a transient hypercoagulable state during which extensive thrombosis regularly develops in distant venous segments containing stagnant blood. Utilizing this technic, single small clots, 4 cm in length, were produced in the jugular veins of 45 dogs. These were released 10 min after their formation and the animal sacrificed 3 min to 28 days after embolization. Heparin, administered preterminally, prevented postmortem clotting. Although intracardiac clots were frequently found, all were smaller than the original thrombus, none was attached to the endocardium, and none was recovered after 4 days. In 27 animals, multiple massive clots (20 - 200 cm in total length) were induced and released from peripheral veins. Again, non-adherent intracardiac clots were frequently found within several hours of embolization. However, among the 16 animals in this group sacrificed 1-67 days after embolization, there were 4 in which thrombi were found adherent to the endocardium of the right auricle or to the leaflets or chordae tendinae of the tricuspid valve. In 2 of these animals sacrificed 7 and 36 days after embolization, there was microscopic evidence of organization of the site of adherence. These findings demonstrate that organizing right-sided endocardial thrombi may be produced in the normal dog by emboli consisting of the animal's own clotted blood released from a peripheral vein. The occurrence of this phenomenon appears, in part, to be a function of the amount of clot released to the circulation.

1612. CURATIVE EFFECT OF RAT BONE MARROW TRANSFUSIONS IN APLASTIC (SEVERE HYPOPLASTIC) ANEMIA IN RATS INDUCED BY MYLERAN, A RADIOMIMETIC CHEMICAL. J. K. Weston, R. E. Maxwell,* Mary Lee,* Joan Finzel* and R. A. Fisken.* Research Lab., Parke, Davis and Co., Detroit, Mich.

Fifty Sprague-Dawley rats were given 20 mg/kg Myleran in 50% dimethylacetamide intravenously. Ten of these served as controls. All died within 2 wk exhibiting severe marrow hypoplasia. Twenty were given $125x10^6$ rat nucleated marrow cells intravenously once, on the 5th day after Myleran administration. The other 20 were given 3 consecutive i.v. marrow injections as above, one each on the 3rd, 4th and 5th days after Myleran administration. Other than 1 death during the 9th wk in the single marrow treatment group (associated with pneumonia) all the animals in the 2 treated groups are apparently in excellent health, have gained weight and demonstrate a normal peripheral blood picture approximately 8 months later. It is concluded that a severe, chemically-induced marrow hypoplasia in rats can be cured by rat marrow transfusions. The utility of this chemically-induced aplastic anemia in rats, both as a bioassay tool to evaluate substances that can ameliorate marrow damage and as an instrument to aid in better understanding marrow hypoplasia, will be pointed out. *(Done under Atomic Energy Comm. contract #AT(11-1)-334.)

1613. DIETARY FAT AND THE PATTERN OF HEPATIC LIPOSIS IN CHOLINE-DEFICIENT MICE. W. Lane Williams, John B. Cardle* and Roland D. Meader.* Dept. of Anatomy, Univ. of Minnesota, Minneapolis.

Mice were fed a choline-deficient diet containing 40% fat. Lard was the fat component in dietary group I. In group II the fat component consisted of 3 synthetic triglycerides (tripalmitin 10%; trimyristin 10%; trihexanoin, 20%). Mice were restricted to these diets when 10 wk of age and were killed 1-30 days later. Fat within cytoplasm of hepatic parenchyma was studied in frozen sections stained with Sudan dyes. In the group (I) fed lard the initial (24 hr) liposis was centrilobular and extended to portal zones within 72 hr. In the triglyceride group (II) initial liposis was restricted to portal zones and increased in these peripheral portions of the lobules. In this group (II) centrilobular liposis was restricted to small globules of fat arranged in an irregular pattern within some cells of the central one-half of some of the lobules. This small amount of fat decreased during the second half (14-30 days) of the experiment. There were no significant weight changes in either dietery group. Cardiovascular lesions were limited to the myocardium of one animal in the triglyceride group.

1614. HISTOGENESIS OF ANTIBODY PRODUCING INTRAOCULAR TRANSPLANTS OF THYMUS IN MICE. William L. Williams, Richard D. Stoner,* and William M. Hale. Dept. of Pathology and Microbiology, School of Biological Sciences, Univ. of Tennessee, Memphis, and Brookhaven Natl. Lab., Upton, N. Y.

Hale and Stoner in 1953 demonstrated the ability of transplants of spleen, lymph node, thymus and Peyer's patch to produce antibodies. The donor mice received a primary stimulus of tetanus toxoid before transplantation. The recipient mice were subjected to 650 rep gamma irradiation just prior to transplantation. The present study concerns the morphological changes which take place in the transplanted lymphoid tissue from 12 hr to 60 days after transplantation. The histology of the transplants at the time of demonstration of antibody production will be stressed. This period will allow a further demonstration of cell types which are capable of producing antibodies as discussed by the authors in the Yale J. Biol. & Med. 28:No. 6, 1956).

1615. INJURY OF THE MOUSE LIVER, PROTECTED FROM CCl_4 INJURY BY SULFAGUANIDINE, AFTER NEMBUTAL TREATMENT. J. Walter Wilson and Elizabeth H. Leduc.* Biological Lab., Brown Univ., Providence, R. I.

Twenty-four hours after CCl_4 administration there is centrolobular necrosis in the liver of the mouse and sleeping time produced by Nembutal is much longer than normal. Mice on a diet containing 2% sulfaguanidine are protected against CCl_4 in that the liver shows no histologically demonstrable injury. Experiments were conducted to find out if, in protected livers, injury not demonstrable histologically may be present. Dosages were as follows: 0.1 ml of a 40% solution of CCl_4 injected subcutaneously, and 0.3 ml of a 0.8% solution of Nembutal in distilled water injected intraperitoneally. The sleeping time was significantly prolonged. That of untreated animals is about 2 hr. Twenty-four hours after CCl_4, with or without sulfaguanidine, this is extended to 8-14 hr. Nembutal alone produces no histologically demonstrable effect on the liver, but after CCl_4 in the sulfaguanidine protected mice, the centrolobular cells become distended with uniform round vacuoles, apparently hydropic. A similar change has

been observed rarely in sulfaguanidine protected $CC1_4$ treated mice without Nembutal, and in a few with Nembutal (2 out of 20 in one experiment) it has not occurred. It can be concluded that in the liver protected against necrosis after $CC1_4$ by sulfaguanidine, there is nevertheless an injury not revealed by histological methods that is still present 24 hr after treatment and which may become demonstrable after Nembutal treatment. The "physiological injury" revealed by the extended sleeping time must involve some imperceptible or incipient "morphological injury".

1616. ANTIBACTERIAL ACTIVITY OF SERUM FROM PATIENTS SHOWING HYPER- AND HYPO-GAMMA GLOBULINEMIA. Don C. Wood (intr. by Russell S. Jones). Fort Douglas VA Hosp., and Dept. of Pathology, Univ. of Utah College of Medicine, Salt Lake City.

A study has been made in man comparing the quantitative relationships between gamma globulin and other antibacterial systems. Sera from patients showing less than 10% gamma globulin, as measured by paper electrophoresis, have been examined for antibacterial potency using a Warburg technique. All of these patients with various diseases had antibacterial titers which at least equalled normal individuals and some had over 2 times the normal value. The presence of large amounts of gamma globulin in serum failed to show consistent increase in antibacterial potency. It would seem apparent, therefore, that some of the antibacterial factors found in serum vary independently of gamma globulin. Besides the complement-dependent properidin system there are heat-stable antibacterial factors that often comprise a large percentage of the total antibacterial property of serum. Both the properidin and the heat-stable (56° C for 30 min) systems vary independently of the gamma globulin level.

1617. EFFECT OF LOCALIZED INFLAMMATION ON ABSORPTION OF PROTEIN. William D. Woods, William Mayer and Charles L. Yuile, (intr. by J. Lowell Orbison). Dept. of Pathology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

In the presence of s.c. turpentine abscess, dogs studied for the synthesis of specific proteins moities, were noted by Charles. L. Yuile et al., to have delayed absorption of proteins when fed by stomach tube. To specifically study this absorption, abscesses of the same type were produced and the dogs were fed whole dog plasma containing C^{14} labeled proteins or human albumins containing tracer amounts of I^{131}. The test meals were not fed until the dogs exhibited the greatest general reaction: temperature elevation, anorexia, and lassitude; and this was concomitant with the greatest local reaction. The immediate period of absorption was followed by repeated sampling of plasma for C^{14} or I^{131} content. Alterations in absorption from the gastro-intestinal trace were indicated by the delayed and reduced plasma levels of C^{14} and I^{131} in the experimental animals. Fecal excretion of C^{14} was not significantly altered except in one dog, in which 24% of the initial dose of C^{14} was recovered. I^{131} levels in the feces were not significantly altered, although I^{131} excretion in the urine was prolonged.

1618. TISSUE HEMOLYTIC SYSTEM OF LIPIDIC NATURE IN MAN. Electra Xefteris* and Mario Stefanini. Saint Elizabeth's Hosp. and Dept. of Medicine, Tufts Univ., Boston, Mass.

Benzene extraction of homogenized blood-free pulp from 7 normal surgical spleens yielded fraction exhibiting strong hemolytic activity against washed normal human erythrocytes. For further purification, benzene extract was dried, redissolved in chloroform, migrated through silicic acid column with chloroform as mobile phase. Product contained cholesterol, fatty acids and neutral fats. Dried material was redissolved in petroleum ether; fatty acids were separated from neutral fats and cholesterol by Bodanski's technic. Fatty acid fraction contained all original hemolytic activity which appeared related primarily to long chain, unsaturated fatty acids. Hemolytic fractions were obtained from many organs, activity being highest in liver; lower in kidney, lung, brain and spleen tissue in this order. Residue of tissue after benzene treatment was extracted with 4:1 chloroform-methanol mixture. Dried extract was redissolved in chloroform, passed through silicic acid column using chloroform as first and methanol as second migrating phase. The latter fraction inhibited activity of the hemolytic agent. Concentration of inhibitory fraction was high in brain, spleen, lung; low in liver and kidney tissue. Free phospholipids from brain, but not from other organs, also exhibited inhibitory activity. Hemolytic activity exceeding normal was found in a majority of pathologic spleens, highest values being obtained in some patients with acquired hemolytic anemia. Inhibitory activity was lower than normal in most instances and usually the higher the hemolytic, the lower was the inhibitory activity of splenic tissue. Thus, many tissues contained a balanced hemolytic system. In pathologic conditions, at least in splenic tissue, hemolytic activity may predominate.

1619. PROTEIN SYNTHESIS AND NUCLEIC ACID ALTERATIONS IN LIVERS OF FASTED AND TURPENTINE-INJURED RATS. L. J. Zeldis, J. K. Ekegren,* J. H. Bloch* and S. C. Madden. Dept. of Pathology, Univ. of California School of Medicine, Los Angeles.

Ribonucleic acid content of liver cells decreases approximately 25% and liver protein nitrogen about 20% during a 24-hour fast in young adult Long-Evans rats. In rats similarly fasted after subcutaneous injection of 1 ml of turpentine, liver RNA is increased 25% and protein nitrogen 18% above values in normal, stock-fed rats. Increases in RNA are found, in livers fractionated in isotonic sucrose, principally in the small particle ("microsome") fraction and in the "fluffy layer". Increases in protein nitrogen occur principally in the supernatant, "fluffy layer" and mitochondrial fractions. RNA/protein nitrogen ratios as well as rates of amino acid incorporation in the "fluffy layer" support the view that the layer is a mixture of microsomes and mitochondria. Histologic changes in the livers of injured rats are those of cloudy swelling without necrosis. Incorporation of large i.v. doses of dl-lysine N^{15} into the liver proteins of injured, normal, and fasted rats has been studied under conditions which yield a constant initial rate of isotope uptake. Incorporation in all animals is most rapid in the microsomal fraction. The net rate of incorporation is increased in injured as compared to normal or fasting rats. The data support the view that increased urinary nitrogen in the injured animal does not derive from a shunt of free amino acids into degradative pathways in the liver. (Supported by Grant No. C1983, National Insts. of Health, PHS.)

AMERICAN INSTITUTE OF NUTRITION

Twenty-first Annual Meeting

CHICAGO, ILLINOIS, APRIL 15-19, 1957

*An asterisk * following an author's name indicates "by invitation"*

1620. GROWTH FACTOR IN MEAT MEAL. C. J. Ackerman* and R. W. Engel. Dept. of Biochemistry and Nutrition, Virginia Polytechnic Inst., Blacksburg.

The effect of meat meal on the growth of rats fed various goitrogenic agents was studied. Weanling Holtzman rats were divided into groups of 8 and each group was fed a complete diet containing one of the following (in per cent): thiouracil 0.1, thiourea 0.1, sulfathiazole 1.0, sulfapyridine 1.0, sulfanilamide 1.0, sulfaguanidine 1.0, sulfamethazine 0.3 and sucrose 1.0. The diet of 4 rats from each group included 10% meat meal and a comparable amount of peanut meal was incorporated into the diet of the remaining 4 rats in each group. After 7 wk, the animals were killed and their thyroid glands removed and weighed. Except for the sulfamethazine, sulfanilamide and sucrose groups, growth was inhibited after the 4th wk in all rats not receiving meat meal. The following represents the average gain in grams per rat during the 4th-7th wk when fed the goitrogenic agent without and with meat meal: thiourea 11, 48; thiouracil 10, 55; sulfamethazine 74, 69; sulfathizaole -4, 47; sulfapyridine 0, 51; sulfanilamide 51, 59; sulfaguanidine 4, 47; sucrose 68, 81. The average thyroid weights as mg/100 gm of body weight, without and with meat meal, were as follows (in same order as above): 11.8, 7.8; 21.1, 17.0; 9.8, 7.9; 29.6, 27.0; 46.7, 49.9; 10.3, 8.5; 33.7, 14.0; 5.3, 4.8. Other experiments indicate that the active principle in meat meal is also found in hog stomach, hog pancreas and the duodenal section of hog stomach.

1621. INOSITOL METABOLISM. Bernard W. Agranoff (intr. by G. M. Briggs). Lab. of Neurochemistry, Natl. Inst. of Neurological Diseases and Blindness, Bethesda, Md.

Labeled inositol was prepared by catalytic tritiation of scylloinosose. Utilization of the myo-inositol-2-H^3 thus formed was examined in the rat in vivo and in vitro. Four hours after the intraperitoneal injection of labeled inositol, radioactivity was measured in ether, alcohol and chloroform-methanol extracts of acid-insoluble tissue fractions. Significant incorporation of tritium was observed in the extracted fractions from liver, kidney, lung, brain and pancreas. The highest specific activity was noted in the alcohol extract of kidney. Tritium was recovered from lipid extracts of tissue slices and broken-cell preparations of kidney as well as liver incubated with inositol-2-H^3 in the presence of glucose, but THO production was observed only in the kidney preparations.

1622. NUTRITIVE VALUE OF RAPESEED OIL. J. C. Alexander* and F. H. Mattson. Research Div., Procter and Gamble Co., Cincinnati, Ohio.

Thomasson (J. Nutrition 56:455, 1955) has reported that rapeseed oil when fed at a level of 50% by weight of the diet causes deaths of all experimental rats in a mean of 17 days and that it has a growth-retarding action at lower levels of intake. In view of the worldwide importance of rapeseed oil as an edible fat, further study of its nutritional value is essential. A 7-wk growth study was weanling male rats has been carried out. Rapeseed oil or soybean oil was fed as 5, 15, 25, 35 or 50% by weight of a purified diet. In contrast to the report of Thomasson, none of the rats died even at the highest dietary level of rapeseed oil. At the 35 and 50% levels of dietary fat, the animals on the diets containing rapeseed oil consumed less feed and grew less than those on comparable diets containing soybean oil. However, at any level of dietary fat there was no difference in caloric efficiency regardless of whether the fat was rapeseed oil or soybean oil. Blood cholesterol values or liver cholesterol levels were similar whether the rats consumed diets containing rapeseed oil or soybean oil. The adrenal cholesterol was increased when rapeseed oil was fed. The magnitude of change was approximately one-eighth of the increase in adrenal cholesterol reported by Carroll (Endocrinology 48:101, 1951) who also found a "marked" increase in liver cholesterol.

1623. NUTRITIVE VALUE AND HARMLESSNESS OF HYDROGENATED FATS. R. E. Alfin-Slater, A. F. Wells,* L. Aftergood* and H. J. Deuel, Jr. Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Southern California, Los Angeles.

Postional and stereoisomers of the unsaturated fatty acids are formed during the hydrogenation of oils. Melnick and Deuel (J. Am. Oil Chemists' Soc. 31:63, 1954) have demonstrated that the fatty acid isomers so produced are not only harmless but of full nutritional value in studies using both microbiological techniques and the growth and metabolic responses of test animals. In this repsect are presented the results of long term studies conducted on rats subsisting for 46 generations on diets with selectively hydrogenated vegetable oils (containing 35% trans fatty acids and 3% linoleic acid isomers) comprising practically the sole source of fat in the diet. The test rats consumed per unit of body weight about 9 times the quantity of fatty acid isomers eaten by the average human assuming that all the visible fat in the diet of the latter is derived from hydrogenated fat. The performance of the test animals

as judged by gain in weight, tibia length, reproduction and lactation, longevity and histopathological examination of the tissues has shown that the hydrogenated fats are completely digestible and of full nutritional value. Nor are the plasma and liver cholesterol values of the animals fed and hydrogenated fat adversely affected. The fatty acid isomers contained in the hydrogenated fat were shown not to be antimetabolites of the essential fatty acids. No deleterious effects were observed as a result of the ingestion of the small amounts of saturated fatty acids present in the hydrogenated fat.

1624. PROTEIN NUTRITION IN THE CAT. J. B. Allison and S. A. Miller.* Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.

The semisynthetic diet for the study of the nutrition of the cat has been improved, the diet producing excellent growth and nitrogen retention when casein or egg proteins were used as protein sources. The nitrogen balance index for casein was high, being approximately 0.9 for both maintenance in the adult and maintenance and growth in the kitten. The data suggest that the sulfur amino acid requirement is relatively less in the cat than in the dog or the rat. The index for wheat gluten was high (0.47) for maintenance in the adult cat but was lower for growth. The data suggest that the lysine requirement for maintenance is lower than for growth. The nitrogen retained for growth and maintenance in the 8-wk-old kitten fed an optimum casein intake was approximately 1.5 gm/day/kg body weight. At 30 wk the nitrogen retained had dropped to 1 gm/day/kg. At 40 wk the retention averaged 0.55 gm/day/kg and at 60 wk it was close to the 0.3-0.35 gm nitrogen/day/kg required for maintenance in the adult cat. Relative values obtained on the beagle dog were 1 gm nitrogen/day/kg body weight at weaning, 0.5 gm/day/kg at 10 wk and 0.2 gm/day/kg at adulthood. The caloric intake of the cat varied from 220 calories/day/kg body weight at time of weaning to 150 calories at 20 wk, to 60-70 calories/day/kg body weight during adulthood.

1625. SAFFLOWER OIL, HYDROGENATED SAFFLOWER OIL AND ASCORBIC ACID EFFECTS ON SERUM CHOLESTEROL IN MAN. Joseph T. Anderson, Francisco Grande* and Ancel Keys. Lab. of Physiological Hygiene, Univ. of Minnesota and Hastings State Hosp., Minneapolis.

Thirty grams of safflower oil or the same oil eslectively hydrogenated, containing, respectively, 23 and 3 gm of linoleic acid (as glycerides), were isocalorically substituted for carbohydrate for 3 wk each in the daily rations of 13 men on a typical American diet providing 98 gm of proteins, 7 gm of linoleic acid, 100 mg of ascorbic acid and 38% calories from fats. At the end of the safflower oil period the average serum cholesterol concentration had fallen by 9.6±4.4 mg % (mean ±SE) while the corresponding value at the end of the period on hydrogenated safflower oil was 5.8±4.8 mg %. Another group of 14 men were studied in an identical experiment except that each man also ingested a supplement of 1000 mg of ascorbic acid. These men showed average decreases of 23.1±4.3 and 8.2±3.5 mg % of serum cholesterol at the ends of the periods on the untreated and the hydrogenated safflower oil, respectively. The difference between the results on the 2 oils in the presence of the ascorbic acid supplement was highly significant with $P = 0.0008$ but when the supplement was omitted the difference was not significant.

1626. EARLY BIOCHEMICAL CHANGES WITH TREATMENT IN KWASHIORKOR. Guillermo Arroyave,* Fernando Viteri,* Moises Behar* and Nevin S. Scrimshaw. Inst. of Nutrition of Central America and Panama, Guatemala.

Laboratory tests during early hospital therapy revealed a remarkable rate of biochemical change in the first few days, not apparent from studies based on weekly examinations. In 8 cases 75,000 μg of vitamin A palmitate in oil given by stomach tube on admission with a test meal 2 hr later produced little effect on the vitamin A serum levels in the 5 subsequent hours (11 μg to 17 μg 100 ml). When the same test was repeated 5 days of milk therapy, a prompt increase of approximately 8-fold in serum vitamin A was obtained (27 μg to 214 μg 100 ml). In 4 cases in which the initial test was omitted, a similar average increase was observed after 5 days of the same therapy (10 μg to 92 μg 100 ml). In these cases, however, the average peak value was significantly lower than when the test dose was also given on admission. Good absorption (10 μg to 87 μg 100 ml) was observed in another case after 4 days and significant, though less marked responses, in 2 of 3 infants after 3 days of therapy. In patients whose hospital treatment was not under research control, responses were negative after 3 days in 6 cases and after 5 days in 3 cases. The values of serum proteins, pseudocholinesterase, alkaline, phosphatase and amylase were also investigated during the 1st week of treatment. Prompt changes occur in these biochemical indexes but their magnitude is frequently obscured by blood volume changes. Examples are presented for both milk and vegetable protein treated cases.

1627. DIET AND FECAL FAT EXCRETION IN TROPICAL SPRUE. Conrado F. Asenjo, R. Rodriguez Molina* and Marta Cancio.* Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Puerto Rico and San Patricio VA Hosp., San Juan.

Six sprue patients were investigated. When they were offered a test diet that supplied 90-95 gm of easily digestible fats per day (actual intake about 80 gm) their fecal fat excretion was above 6 gm. When the fat intake was reduced to 10 gm/day the streatorrhea disappeared and the daily fecal fat excretion was of the same order of magnitude as that of normal subjects, that is, 4 gm or less. Under the conditions of our experiment and using trials that lasted 12 days, the presence or absence of gluten in the low-fat diets did not affect the so-called metabolic fecal fat excretion (fecal fat of endogenous, tissue and bacterial origin). In this respect the steatorrhea of spure seems to be different from that of celiac disease. The daily excretion of volatile fatty acids was also investigated in a limited number of sprue and normal subjects. It seems to be independent of the type of diet used (normal mixed diet, high fat test, low fat, or low fat plus gluten) and also of the type of subject (sprue or normal). Formic and/or acetic, propionic and valeric acids were characterized by paper chromatography in the water soluble fraction. The insoluble volatile acid fraction is composed of only one acid which so far has not been characterized. (Supported by a Public Health Service grant).

1628. DIETARY FATS AND TIME IN PRODUCTION OF EXPERIMENTAL OBESITY. Joseph J. Barboriak (intr. by W. A. Krehl). Nutrition Lab., Yale Univ., New Haven, Conn.

Experiments, ranging in duration from 44 days to almost 2 yr, were conducted starting with weanling male rats. The following fats were evaluated at a level of 62% of the diet: butter fat, lard, Crisco, margarine, corn oil, coconut oil, and cottonseed oil.

An isocaloric high-carbohydrate diet was fed to the controls. Groups receiving corn oil, coconut oil and cottonseed oil exhibited lower initial weight gains (i.e. in 21 days they gained 50, 70 and 47 gm, respectively, as compared with 96 gm for the controls). In this same period weight gains of the lard, Crisco and butter fat groups were 127, 135 and 123 gm, respectively. After an additional 3 wk, however, the (oil-fed) groups had equaled or surpassed the weight gain of the control group. After about 2 mo, a definite order of weight gain was established and this order persisted. The order of weight gain from greatest to least was directly related to the degree of saturation of the fats tested from greatest to least. An exception was coconut oil, which though highly saturated, allowed only moderate weight gains. Finally, the animals gained the most weight on diets containing Crisco or lard, then followed butter fat or margarine, then corn oil, coconut oil and cottonseed oil. A substantial number of animals on high fat intakes developed extreme obesity with weight gains in excess of 1200 gm. The animals at autopsy were examined for gross pathology and histological sections were made for review of microscopic pathology.

1629. NUTRITIONAL SIGNIFICANCE OF COPROPHAGY. Richard H. Barnes, Grace F. Fiala,* Bette J. McGehee* and Ann M. Brown.* Grad. School of Nutrition, Cornell Univ., Ithaca, N. Y.

Numerous attempts have been made to prevent coprophagy in experimental animals in nutritional studies. The standard practice for minimizing coprophagy has been the use of raised wire screens, even though it is well known that rats and other experimental animals, though maintained on raised screens, will eat their feces. A specially designed cup which fits over the tail and is held to the rat by a collar placed on the tail has been found to be an effective way of completely preventing coprophagy. Such rats, when maintained on a highly purified diet, grow at a rate that is approximately 10% below their controls. Feces are solid in color while in the controls a mottled appearance is usually found. When coprophagy is prevented approximately 10% less dry weight of feces is excreted per day and voluntary food intake is about 10% less than in normal controls. Normal rats that are fed a highly purified diet that is lacking in vitamin K do not develop symptoms of vitamin K deficiency. However, there is a slightly prolonged prothrombin time of rats in which coprophagy is prevented, indicating a minimal K deficiency. With the highly purified K deficient diet that has been employed in these studies, 2% succinylsulfathiazole does not affect the growth rate and causes only a slight increase in prothrombin time of rats that are maintained on raised wire screens. On the other hand, the same diet fed to rats in which coprophagy is prevented results in about a 20% decrease in growth rate and an enhanced prolongation of prothrombin time.

1630. ALTERATIONS IN INTERMEDIARY METABOLISM IN PREGNANCY. George H. Beaton and Doris M. Curry (intr. by E. W. McHenry). Dept. of Public Health Nutrition, Univ. of Toronto, Canada.

Significant alterations in the intermediary metabolism of protein have already been reported for the pregnant rat. The evidence suggested a role of anterior pituitary growth hormone in bringing about these changes. Pregnant rats also exhibit an increased catabolism of fat as evidenced by a marked elevation of fasting blood ketone bodies. Significant changes in the levels of blood metabolites and tissue enzymes with carbohydrate metabolism will be reported. Pregnancy represents a period of major metabolic adaptations involving more than the simple withdrawal of materials to the growing fetus.

1631. AMINO ACID SUPPLEMENTATION OF OATS. D. A. Benton and J. N. Tang (intr. by Robert MacVicar). Dept. of Agricultural Chemistry, Oklahoma A. and M. College, Stillwater.

Differences were observed in the weight gains of weanling rats when different varieties of oats were fed as the source of protein. A supplement of lysine increased the weight gained by rats fed oats of high nutritive value. A supplement of methionine alone reduced the weight gain and a supplement of threonine alone was without effect. Supplements of lysine and methionine or lysine and theronine caused no more weight gain than lysine alone. A supplement of 0.6% L-lysine, 0.5% DL-methionine and 0.75% DL-threonine caused an increase in the weight gain beyond that obtained with lysine alone or in combination. Although this last diet contained only 2.1% nitrogen, the weight gain was the same as that of a group fed 20% casein. A comparison was made of the growth responses to the amino acid supplements with cat samples of relatively high and low nutritive value. A lysine supplement caused a greater growth response with oats of high nutritive value. This increased the difference in weight gain between the groups fed the difference oats. A supplement of lysine, ethionine and threonine to oats of high and low nutritive value resulted in large but significantly different gains. Consecutive extractions of the oats fed in these trials with water, 10% NaCl, 70% ethanol and 0.2% NaOH failed to indicate differences.

1632. TORULA YEAST AND VITAMIN E DEFICIENCY IN THE CHICK. J. G. Bieri, C. J. Pollard* and G. M. Briggs. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

The production of classical vitamin E deficiency symptoms in the chick requires a stress in the form of unsaturated fat. In the absence of such fat, symptom-free chicks can be grown on a purified vitamin E-free diet for prolonged periods even though serum tocopherols are zero and tissue tocopherols are very low (Bieri et al. Poultry Sci. 35:1134, 1956). Scott et al. (J. Nutrition 56:387, 1955) have shown that the occurrence of exudative diathesis in chicks fed diets high in Torula yeast is independent of added fat. We have found that on a similar fat-free diet containing 60% of alcohol extracted Torula yeast deficiency symptoms also occur. It is apparent that the presence of unsaturated fat is not necessary for production of symptoms under these conditions. Experiments in which -tocopherol acetate was added to diets containing Torula yeast or soy portein suggest that the utilization of vitamin E is decreased in chicks fed Torula yeast. Two groups of chicks were fed diets containing 4% of vitamin E-free lard and 100 mg of α-tocopherol acetate/kg. Diet 1 contained 60% of Torula yeast while diet 2 contained 28% of soy protein. After 14-16 days, serum tocopherol levels were: diet 1, 827±191; diet 2, 2069±438. Liver stores were altered similarly. The effect of various proteins and of amino acid mixtures on the symptoms produced by Torula yeast, and the relationship between tissue tocopherol content and the occurrence of symptoms, will be discussed.

1633. ARGININE "TOXICITY" IN RATS FED RAW SOYBEAN OIL MEAL RATIONS. Raymond Borchers. Dept. of Biochemistry and Nutrition, Univ. of Nebraska, Lincoln.

The reduced growth rate and appearance of rats

fed raw soybean oil meal rations (when compared with autoclaved meal) suggested a possible arginine deficiency. Supplementation of the raw soybean ration with arginine by injection or by 0.5% addition to the rationdid not effect a response. However, the addition of 2% L-arginine hydrochloride resulted in a 25% decrease in the growth rate. Similar addition to the autoclaved soybean oil meal ration showed no such effect. Interrelationships between the growth inhibitor of raw soybeans and arginine "toxicity," arginine metabolites, other amino acids and antibiotics will be presented.

1634. EFFECT OF ANTIBIOTICS ON FOLIC ACID DEFICIENT CHICKS. J. E. Braham,* H. R. Bird and C. A. Baumann. Depts. of Poultry Husbandry and Biochemistry, Univ. of Wisconsin, Madison.

Penicillin and chlortetracycline do not appear to produce consistent effects in chick diets low in folic acid. Recorded effects varied from a substantial increase in growth (J. Nutr. 52:627, 1954) to a depression in growth (Ibid. 51:355, 1953). In the present studies, on a practical ration low in folic acid, procaine penicillin produced a 28% average increase in growth and chlortetracycline 16%. For 12 series on a semi-synthetic ration, average increase in weight was 6% when procaine penicillin was added to diets containing 0.25°0.35 mg of folic acid/kg, while chlortetracycline produced no increase in growth. In another series with a diet containing 0.25 mg of folic acid/kg, responses to procaine penicillin and chlortetracycline were 8% and 2% respectively, but responses to streptonivicin, chloromycetin and bacitracin at levels of 35 ppm were respectively, 49, 35 and 16%. Analyses of livers showed that the effects of procaine penicillin and chlortetracycline upon folic acid storage were roughly comparable to effects upon growth. In a typical experiment with a diet containing 0.35 mg of folic acid/kg the following results were obtained in μg of folic acid/gram of wet tissue: control, 2.77; procaine penicillin-supplemented, 4.54; chlortetracycline-supplemented, 3.10. With a diet containing 0.50 mg of folic acid/kg the control group gave a value of 5.09 as compared to 5.18 and 5.41 μg folic acid/gm of wet tissue for procaine penicillin and chlortetracycline respectively.

1635. EFFECT OF LIME-TREATMENT ON SOME CHARACTERISTICS OF CORN PROTEINS. Ricardo Bressani (intr. by Robert L. Squibb). Inst. of Nutrition of Central America and Panama, Guatemala.

It appears probable that the pellagragenic property of corn is due to the poor amino acid balance of the largest protein fraction, zein. Comparison of the solubility of the proteins of corn with those of tortillas prepared from it by lime and heat treatment showed a reduction in the percentage of nitrogen in the fractions which were water-soluble (16.7% to 9.4%), salt-soluble (14.4% to 7.0%), alcohol-soluble (30.7% to 6.4%) and sodium hydroxide-soluble (28.4% to 15.5%). The marked decrease in the alcohol-soluble fraction which represents a decreased solubility of zein, may be responsible for a better amino acid balance in tortillas than corn. Microbiological assays of the 10 essential amino acids plus cystine and tyrosine calculated on the basis of 16 gm of nitrogen showed significant losses due to the process of preparing tortillas of arginine (13%), leucine (23%) and cystine (18%); a slight increase in several of the amino acids was explained by the cumulative losses of carbohydrate (5%) and nonprotein nitrogen (3%) in the tortilla process. Enzymatic digestion showed total and alpha-amino nitrogen to be released more rapidly from corn than from tortillas during the first 12 hr of hydrolysis. After 48 hr the quantities released were similar. Several of the eseential amino acids also appear to be more readily available from corn than from tortilla. Whichever hydrolysis was employed, the niacin and tryptophan contained in corn and tortillas as determined microbiologically were equally well released.

1636. ENZYMATIC CONVERSION OF D-GLUCURONATE TO L-ASCORBATE IN ANIMAL TISSUES. C. Publitz,* A. P. Grollman* and A. L. Lehninger. Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Md.

Earlier work in this laboratory has demonstrated that the net conversion of D-glucuronate to L-ascorbate in rat liver extracts requires DPN, TPN and Mn^{++} This report deals with the characterization of the enzymatic steps involved. In the 1st step, D-glucuronate is reduced specifically by TPNH to L-gulonate in a reversible reaction; the corresponding γ-lactones react only after hydrolysis. The partially purified enzyme from pig kidney also reduces D-galacturonate. In the 2nd step, L-gulonate is oxidized to an unidentified intermediate, presumably 3-keto-L-gulonate, in a reaction involving specifically DPN andMn^{++}. L-gulono-γ-lactone is attacked after hydrolysis. The reaction products depend on experimental conditions. Under some conditions xylulose and CO_2 are the only reaction products and are presumably formed by decarboxylation of 3-keto-L-gulonate. However, under other circumstances, L-ascorbate is formed in a 3rd enzymatic step, presumably by enolization and lactonization of 3-keto-L-gulonate. The dehydrogenases catalyzing the first 2 steps described are present in liver and kidney of all mammals tested; the 3rd step of the sequence is involved in the failure to form ascorbic acid in toose species requiring dietary ascorbic acid. (Supported by grants from the National Vitamin Fndn. and the Nutrition Fndn., Inc.)

1637. EFFECTS OF SUBLETHAL DOSES OF X-IRRADIATION DURING PREGNANCY ON REPRODUCTIVE PERFORMANCE OF THE RAT. Amber L. S. Cheng,* R. E. Alfin-Slater and B. H. Ershoff. Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles.

Rats raised to maturity on a natural food ration were subjected to a single sublethal dose of x-irradiation (100, 200 or 300 r) prior to breeding and on the 2nd, 7th, 12th and 16th day of gestation. The effects of such treatment has been contrasted with that of unirradiation controls in regard to the number of litters cast, the number of young born, the weight of the litters at birth, the weight of the young at graded periods of lactation and the number of litters and percentage of each litter surviving the weaning period. (Supported by Contract No. AT (11-1)-113 from the Atomic Energy Comm.)

1638. HEPATIC RESPONSIVENESS TO GLUCAGON. Robert E. Clancy,* Theodore B. Van Itallie, David H. Spodick,* Charles E. Wilder* and David Littmann.* Med. Service and Research Lab., VA Hosp., West Roxbury and Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

To obtain further information about hepatic responsiveness to glucagon administration, studies on human subjects without liver disease were performed, utilizing catheterization of an hepatic vein, a formoal artery and an antecubital vein. Glucagon was administered by single intravenous injection or by constant infusion. Blood glucose and serum inorganic phosphate levels

were determined on samples drawn from all 3 sites before, during and after glucagon administration. When glucagon was administered by constant infusion, liver output of glucose usually reached a peak in 10 min and then tended to diminish even though glucagon administration continued at the same rate for an hour thereafter. When glucagon was given by single injection at a time when the blood sugar was already elevated a) by an injection of glucagon 30 min previously, or b) by i.v. glucose infusion, hepatic output of glucose was appreciably diminished. When a 2nd injection of glucagon was given 60 min after a 1st injection (the blood sugar having returned to control levels), the liver output of glucose was approximately the same as after the 1st injection. Peripheral blood sugar changes after glucagon administration almost always showed a time lag compared with hepatic vein glucose levels. Measurement of peripheral arteriovenous glucose differences failed to disclose enhancdment by glucagon of peripheral glucose uptake anticipated from earlier reports. Evidence was obtained suggesting that the liver removes inorganic phosphate from blood during glucagon-induced glycogenolysis.

1639. ROLE OF LIPOPROTEIN LIPASE IN INTRAVASCULAR CLEARING OF FAT IN HUMAN SUBJECTS. W. W. Cleland and J. M. Iacono (intr. by Carl J. Koehn). U.S. Army Med. Nutrition Lab., Denver, Col.

At intervals during the infusion of intravenous fat emulsion (Lipomul) blood samples were obtained and clearing factor (lipoprotein lipase) was measured by determining the glycerol liberated in 4 hr under conditions where the reaction was zero order. The fasting level was 0-2 U (μg glycerol/ml serum/hr). The activity was highest at the end of the infusion (5-20 U), and dropped as fat disappeared from the blood. The activity was roughly proportional to the level of fat in the blood; thus, persons clearing fat slowly from the blood developed the higher levels of clearing factor and those clearing rapidly, the lower. The 6 hospitalized patients cleared the fat more rapidly and had lower levels of the lipase than the 6 healthy subjects. Comparison of the measured activities with the actual rates of clearing in vivo showed that the enzyme as measured could account for the clearing of only 5% of the fat actually removed from the blood. It is postulated that the clearing factor in tissues rather than circulating clearing factor is primarily responsible for clearing and that the need for the fat as an energy source determines, to some extent, the rate at which the fat is removed from the blood.

1640. CHOICE OF CONCENTRATION OF ACIDIC DRINKING FLUIDS BY RATS. Willard G. Clements* and Gerald J. Cox. Research Dept., School of Dentistry, Univ. of Pittsburgh, Pittsburgh, Pa.

Rats restricted for 4 wk at weaning to 0.05 M acids as the sole source of drinking fluids, showed significant increase of ratio of submandibular salivary gland weights to body weights. Similar increases were shown by rats exceeding 12 mo of age. However, the experimental rats tended to ose weight in comparison with water fed controls, indicating dislike for the acidic fluids. When the concentration of the acids, citric and lactic, were reduced to 0.025 M and 0.0125 M, the ratios were increased more by the less concentrated acids. Diminishing concentrations of acids were supplied from 2, 3 or 4 constant level, automiatic burette fed drinking tubes to 2 adult rats in single cages under constant temperature conditions. From a series of successive dilutions of 0.05 M acids with equal volumesof water, preference was for the more dilute acid to the level of 0.00156 M.

1641. MOLYBDENUM IN CHICK AND POULT AND IN AVIAN NUTRITION. J. R. Couch, A. A. Kurnick,* B. L. Reid* and R. L. Svacha.* Depts. of Poultry Science and Biochemistry and Nutrition, Texas A. and M. College System, College Station.

Molybdenum has been found to stimulate the growth of chicks and pults at 4 wk of age when added to a purified diet consisting of glucose monohydrate, water extracted soybean portein, plus vitamin and mineral levesl though to be adequate. These studies indicate that a portion of the growth increase obtained with the ash of unidentified growth factor sources may be attributed to the molybdenum in such sources. The xanthine dehydrogenase activity of liver and intestinal tissues from chicks and pults was increased significantly when the basal diet was supplemented with molybdenum, distillers dried solubles or the ash of distillers dried solubles. A positive correlation of 0.955 was found between liver molybdenum and xanthine dehydrogenase activity. The molybdenum content of the bones and livers was increased when the diet was supplemented with molybdenum (0.05 ppm), 3% distillers dried solubles or the ash of this product; such supplementation had no effect on the percentage bone ash of birds so fed. The availability of molybdenum in natural supplements may be an important factor in the correlation of the growth promoting and tissue dehydrogenase activity obtained with these products. Molybdenum as $Na_2MoO_4 \cdot 2\ H_2O$ has been found to produce a significant increase in the growth of chicks fed a diet containing corn, milo, soybean oil meal, corn gluten meal and with added minerals and vitamins in chicks maintained on litter at 10 wk of age.

1642. EFFECT OF VITAMIN D ON URINARY FREE AMINO ACIDS IN THE RAT. J. W. Cramer,* G. W. Engstrom* and H. Steenbock. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

It has been reported that in human rickets amino acids are excreted in the urine in excessive amounts as the result of a diminished tubular reabsorption of certain amino acids. In some way as yet undetermined, treatment and healing of rickets with vitamin D restores the power of reabsorption of these amino acids. In the present study the rat was used as the experimental animal to determine if this species shows an aminoaciduria in rickets and if treatment with vitamin D would correct it. The effect of an increase in protein intake on the aminoaciduria was also studied, as well as the effect of adding certain amino acids to the diet in excessive amounts. Determinations of the amino acid levels in the blood, kidney and liver were also made. The amino acids were determined by the ninhydrin method of Van Slyke. Young rats were used in all of the experiments and a semisynthetic low-P rachitogenic diet was fed. The aminoaciduria observed in rickets in children was found to occur in rats when fed the rachitogenic diet. This was reduced by the administration of vitamin D. Even when the rats excreted large amounts of amino acids, as was induced by doubling the protein intake, vitamin D reduced the aminoaciduria. Vitamin D also reduced it when the rats were fed large amounts of crystalline amino acids.

1643. BIOCHEMICAL EFFECTS OF VITAMIN E DEFICIENCY IN MONKEYS. Paul L. Day, Douglas E. Young* and James S. Dinning. Dept. of Biochemistry, Univ. of Arkansas School of Medicine,

fed raw soybean oil meal rations (when compared with autoclaved meal) suggested a possible arginine deficiency. Supplementation of the raw soybean ration with arginine by injection or by 0.5% addition to the rationdid not effect a response. However, the addition of 2% L-arginine hydrochloride resulted in a 25% decrease in the growth rate. Similar addition to the autoclaved soybean oil meal ration showed no such effect. Interrelationships between the growth inhibitor of raw soybeans and arginine "toxicity," arginine metabolites, other amino acids and antibiotics will be presented.

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were determined on samples drawn from all 3 sites before, during and after glucagon administration. When glucagon was administered by constant infusion, liver output of glucose usually reached a peak in 10 min and then tended to diminish even though glucagon administration continued at the same rate for an hour thereafter. When glucagon was given by single injection at a time when the blood sugar was already elevated a) by an injection of glucagon 30 min previously, or b) by I.V. glucose infusion, hepatic output of glucose was appreciably diminished. When a 2nd injection of glucagon was given 60 min after a 1st injection (the blood sugar having returned to control levels), the liver output of glucose was approximately the same as after the 1st injection. Peripheral blood sugar changes after glucagon administration almost always showed a time lag compared with hepatic vein glucose levels. Measurement of peripheral arteriovenous glucose differences failed to disclose enhancdment by glucagon of peripheral glucose uptake anticipated from earlier reports. Evidence was obtained suggesting that the liver removes inorganic phosphate from blood during glucagon-induced glycogenolysis.

1639. ROLE OF LIPOPROTEIN LIPASE IN INTRAVASCULAR CLEARING OF FAT IN HUMAN SUBJECTS. W. W. Cleland and J. M. Iacono (intr. by Carl J. Koehn). U.S. Army Med. Nutrition Lab., Denver, Col.

At intervals during the infusion of intravenous fat emulsion (Lipomul) blood samples were obtained and clearing factor (lipoprotein lipase) was measured by determining the glycerol liberated in 4 hr under conditions where the reaction was zero order. The fasting level was 0-2 U (μg glycerol/ml serum/hr). The activity was highest at the end of the infusion (5-20 U), and dropped as fat disappeared from the blood. The activity was roughly proportional to the level of fat in the blood; thus, persons clearing fat slowly from the blood developed the higher levels of clearing factor and those clearing rapidly, the lower. The 6 hospitalized patients cleared the fat more rapidly and had lower levels of the lipase than the 6 healthy subjects. Comparison of the measured activities with the actual rates of clearing in vivo showed that the enzyme as measured could account for the clearing of only 5% of the fat actually removed from the blood. It is postulated that the clearing factor in tissues rather than circulating clearing factor is primarily responsible for clearing and that the need for the fat as an energy source determines, to some extent, the rate at which the fat is removed from the blood.

1640. CHOICE OF CONCENTRATION OF ACIDIC DRINKING FLUIDS BY RATS. Willard G. Clements* and Gerald J. Cox. Research Dept., School of Dentistry, Univ. of Pittsburgh, Pittsburgh, Pa.

Rats restricted for 4 wk at weaning to 0.05 M acids as the sole source of drinking fluids, showed significant increase of ratio of submandibular salivary gland weights to body weights. Similar increases were shown by rats exceeding 12 mo of age. However, the experimental rats tended to ose weight in comparison with water fed controls, indicating dislike for the acidic fluids. When the concentration of the acids, citric and lactic, were reduced to 0.025 M and 0.0125 M, the ratios were increased more by the less concentrated acids. Diminishing concentrations of acids were supplied from 2, 3 or 4 constant level, automiatic burette fed drinking tubes to 2 adult rats in single cages under constant temperature conditions. From a series of successive dilutions of 0.05 M acids with equal volumesof water, preference was for the more dilute acid to the level of 0.00156 M.

1641. MOLYBDENUM IN CHICK AND POULT AND IN AVIAN NUTRITION. J. R. Couch, A. A. Kurnick,* B. L. Reid* and R. L. Svacha.* Depts. of Poultry Science and Biochemistry and Nutrition, Texas A. and M. College System, College Station.

Molybdenum has been found to stimulate the growth of chicks and pults at 4 wk of age when added to a purified diet consisting of glucose monohydrate, water extracted soybean portein, plus vitamin and mineral levesl though to be adequate. These studies indicate that a portion of the growth increase obtained with the ash of unidentified growth factor sources may be attributed to the molybdenum in such sources. The xanthine dehydrogenase activity of liver and intestinal tissues from chicks and pults was increased significantly when the basal diet was supplemented with molybdenum, distillers dried solubles or the ash of distillers dried solubles. A positive correlation of 0.955 was found between liver molybdenum and xanthine dehydrogenase activity. The molybdenum content of the bones and livers was increased when the diet was supplemented with molybdenum (0.05 ppm), 3% distillers dried solubles or the ash of this product; such supplementation had no effect on the percentage bone ash of birds so fed. The availability of molybdenum in natural supplements may be an important factor in the correlation of the growth promoting and tissue dehydrogenase activity obtained with these products. Molybdenum as $Na_2MoO_4 \cdot 2\ H_2O$ has been found to produce a significant increase in the growth of chicks fed a diet containing corn, milo, soybean oil meal, corn gluten meal and with added minerals and vitamins in chicks maintained on litter at 10 wk of age.

1642. EFFECT OF VITAMIN D ON URINARY FREE AMINO ACIDS IN THE RAT. J. W. Cramer,* G. W. Engstrom* and H. Steenbock. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

It has been reported that in human rickets amino acids are excreted in the urine in excessive amounts as the result of a diminished tubular reabsorption of certain amino acids. In some way as yet undetermined, treatment and healing of rickets with vitamin D restores the power of reabsorption of these amino acids. In the present study the rat was used as the experimental animal to determine if this species shows an aminoaciduria in rickets and if treatment with vitamin D would correct it. The effect of an increase in protein intake on the aminoaciduria was also studied, as well as the effect of adding certain amino acids to the diet in excessive amounts. Determinations of the amino acid levels in the blood, kidney and liver were also made. The amino acids were determined by the ninhydrin method of Van Slyke. Young rats were used in all of the experiments and a semisynthetic low-P rachitogenic diet was fed. The aminoaciduria observed in rickets in children was found to occur in rats when fed the rachitogenic diet. This was reduced by the administration of vitamin D. Even when the rats excreted large amounts of amino acids, as was induced by doubling the protein intake, vitamin D reduced the aminoaciduria. Vitamin D also reduced it when the rats were fed large amounts of crystalline amino acids.

1643. BIOCHEMICAL EFFECTS OF VITAMIN E DEFICIENCY IN MONKEYS. Paul L. Day, Douglas E. Young* and James S. Dinning. Dept. of Biochemistry, Univ. of Arkansas School of Medicine,

Little Rock.

Experiments were conducted on young normal rhesus monkeys, vitamin E-deficient monkeys and on monkeys rendered deficient in vitamin E and then recovered with α-tocopherol acetate. The animals were injected with sodium formate-C^{14} and killed after 4 hr. The creatine content of skeletal muscle and heart and the concentrations and specific activities of nucleic acids from a veriety of tissues were determined. The tissues most drastically affected by vitamine E deficiency were skeletal muscle and bone marrow. The deoxyribonnucleic acid (DNA) concentration of skeletal muscle was elevated in the deficient animals and restored to normal in the recovered animals. Vitamin E deficiency did not affect the ribonucleic acid (RNA) concentration of monkey skeletal muscle. Bone marrow from vitamine E-deficient monkeys exhibited greatly increased concentrations of both RNA and DNA and an elevated RNA/DNA ratio. Treatment of the deficient animals with α-tocopherol reversed these changes. The incorporations of formate-C^{14} into the nucleic acids of both skeletal muscle and bone marrow was greatly increased in the vitamine E-deficient monkeys. Therapy with vitamin E completely reversed this effect in bone marrow but only partially reversed the effect in skeletal muscle. The creatine content of skeletal muscle from the deficient animals was reduced to approximately 50% of normal and again treatment of the deficient animals with α-tocopheraol did not completely restore muscle creatine concentrations to normal. Vitamin E deficiency did not markedly affect the concentration of heart creatine.

1644. PROTEIN REQUIREMENTS OF CHINOOK SALMON AT TWO WATER TEMPERATURES. Donald C. DeLong,* John E. Halver* and Edwin T. Mertz. Salmon Nutrition Lab., U.S. Fish and Wildlife Service, Cook, Wash. and Dept. of Biochemistry, Purdue Univ., Lafayette, Ind.

In order to find a suitable protein level for determining minimum amino acid requirements (Fed. Proc. 15:548, 1956), total nitrogen requirements have been investigated. Hatchery Chinook salmon (Oncorhynchus tschawytacha) were divided into 5 lots of 200 each, one lot per trough, average weight of individuals per lot, 1.5 gm. All lots were fed diets containing amino acid mix, casein and gelatin with a balance of essential amino acids similar to whole egg protein, also dextrin, corn oil, cod liver oil, minerals, carboxymethylcellulose, vitamins and water. The 5 lots received, respectively, rations containing 13, 26, 39, 52 and 65% of total protein, dextrin replacing protein to keep the rations approximately isocaloric. Water temperature was maintained at 43°-47°F. At the end of 10 wk, maximum gain was observed at the 39% protein level. A 2nd experiment at the same temperature with 150 fish per lot, average weight 5.8 gm with 9 protein levels at 5% increments from 25% protein to 65% protein again gave maximum gains at bout 40% total protein. The above two trials were duplicated at a water temperature of 58°F, using fish with average weight of 2.6 and 5.7 gm, respectively. In the 1st trial maximum gains were observed at 65% protein, in the 2nd, at 55% and 65% protein. These data suggest that the ability of salmon to tutilize dietary protein for growth is a function of environmental temperature, higher water temperature permitting more efficient utilization of high protein diets.

1645. AMINO ACID IMBALANCE IN LOW PROTEIN DIETS. P. D. Deshpande* and C. A. Elvehjem, Dept. of Biochemistry, Univ. of Wisconsin, Madison.

The earlier observation that a partial deficiency of threonine is produced in young rats fed on a 9% casein diet containing choline and supplemented with methionine and tryptophan led to the study of the effect of amino acid imbalance on growth and liver fat in rats fed other proteins. Although 9% of beef blood fibrin under similar conditions supported about the same rate of gorwth as 9% casein, fat did not accumulate in the liver. Therefore, in the present investigation diets containing only 6% of fibrin were used. When young rats were fed this diet, the addition of 0.4% DL-methionine caused growth retardation but fat did not accumulate in the liver. Supplementation with histidine, phenylalanine, leucine, isoleucine and valine overcame the adverse effect of methionine and stimulated growth but at the same time fatty infiltration of the liver occurred. When any one of the above amino acids was omitted growth was inferior to that obtained when all 6 amino acids were included. Very little growth stimulation occurred with this mixture when methionine was omitted. The results suggest that although addition of methionine to the basal diet created an imbalance which was much more complex in nature than that obtained in previous studies with casein diets, methionine was limiting for maximum growth when other amino acids were included. The role of these individual amino acids in correcting the imbalance and also the effect of additional lysine and threonine on the accumulation of liver fat will be given in greater detail.

1646. CREATINE METABOLISM IN VITAMIN E DEFICIENCY. James S. Dinning and Coy D. Fitch.* Dept. of Biochemistry, Univ. of Arkansas School of Medicine, Little Rock.

Normal and vitamin E-deficient rabbits were injected with glycine-2-C^{14} and killed after varying time intervals. The concentration and specific activities of kidney glycocyamine, liver creatine, skeletal muscle creatine and heart craetine were determined. Vitamin E deficiency led to an elevated concentration of liver creatine, to a reduced concentration of skeletal muscle creatine and was without effect on the concentrations of kidney glycocyamine or heat creatine. The conversion of kidney glycocyamine to liver creatine and the incorporation of liver creatine into skeletal muscle were increased by factors of from 4 to 10 in the vitamin E-deficient rabbits. The turnover of heart creatine was unaffected by vitamin E deficiency. The results show that vitamin E deficiency in the rabbit leads to an increased rate of creatine synthesis and to an increased turnover of skeletal muscle creatine.

1647. SULFHYDRYL CHANGES IN MOUSE SKIN DURING TREATMENT WITH CERTAIN CARCINOGENS. Joseph A. DiPaolo and T. F. Niedbala (intr. by Fred Rosen). Roswell Park Mem. Inst., Buffalo, N. Y.

Several hundred synthetic carcinogens of a wide variety of chemical types are now available. In no case is the mechanism of action understood, though in recent years a few clues have emerged. Crabtree (Brit. Med. Bull. 4:345, 1947) has suggested that the primary step involved in the production of skin tumors with a standard carcinogen is the fixation of the carcinogen to the cell through combination with free sulfhydryl groups. Others have also demonstrated that carcinogenic hydrocarbons react with RSH groups. The total sulfydryl content of mouse epidermis and dermis was found amperometrically and expressed as mg % of -SH. Animals received one painting of 0.02 ml of an acetone solution containing 1,2,5,6-dibenzanthracene, 7,12-dimethylbenzanthracene, or a combination of the two carcinogens. The animals were

killed at fixed intervals from 1/2 hr to 5 days. In general, the shape of the amperometric curve of the total sulfhydryl content of the epidermis and dermis was the same, although the dermis contained approximately half that of the epidermis. Usually at the 1st hr there was an increase over the control and 1/2-hr sample. This was followed by an appreciable drop about an hour later and a subsequent return to normal.

1648. TOCOPHEROL AND OTHER LIPIDES IN ELECTRON TRANSPORT. Kenneth O. Donaldson and Alvin Nason (intr. by E. V. McCollum). McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.

Tocopherol has been implicated as a possible cofactor in the DPN - and succinate - cytochrome c reductases from rat muscle and beef hear (Nason and Lehman. J. Biol. Chem. 222:511, 1956). Enzyme activity, after isooctane extraction, is restored by addition of tocopherol or more effectively by the lipide residue obtained from the isooctane. Upon HCl-ascorbic acid reduction of the hydrlyzed concentrated lipide residue from rat muscle enzyme, it can now be shown that some tocopherol is present, most of it presumably as tocopherylquinone, as already demonstrated by Slater (Nature 177:1181, 1956) for a Keilin-Hartree heart muscle preparation. While the lipide residue tocopherol concentration which represents only 5% of the total tocopherol of the rat particulate enzyme (5 micromoles per gm protein) is too small to restore activity of the isooctane-extracted enzyme, it is possible that this small amount of tocopherol was originally effective at the active enzyme sites. The crude lipide residue contains another factor which has been purified 200-fold from beef heart and is 20 times as active as tocopherol mole per mole. Alkaline hydrolysis completely destroys this lipide cofactor whereas tocopherol reamins active. By means of infrared studies, chemical derivatives and chromatographic and countercurrent distribution techniques, the lipide material has been tentatively identified as a triglyceride with steartate, palmitate and oleate components. Milk, butter, oleomargarine, corn oil and certain synthetic glycerides have some activity. The possible relationship between tocopherol and the lipide cofactor and their possible role in electron transport will be discussed.

1649. INFLUENCE OF SOURCE AND LEVEL OF DIETARY NITROGEN ON NITROGEN BALANCE AND CARCASS COMPOSITION IN THE ADULT RAT. Jacqueline L. Dupont,* Mary W. Marshall* and Madelyn Womack. Human Nutrition Research Branch, U.S. Dept. of Agriculture, Washington, D.C.

Adult male rats (av. wt. 370 gm) were fed diets containing different levels of lactalbumin or of amino acids, with the nitrogen intake of some groups increased by addition of diammonium citrate. Nitrogen balances were determined at intervals, and, after 6 wk of feeding, livers and carcasses were analyzed for fat and protein. The animals were in approximate nitrogen equilibrium when fed 15 gm/day of a 4% lactalbumin diet with sucrose as the carbohydrate and nitrogen balances were not significantly altered when the nitrogen intake was increasec by addition of diammonium citrate. Nitrogen balances were improved by substituting cornstarch for sucrose and further improved by increasing the percentage lactalbumin in sucrose-containing diets. The greatest percentage retention of nitrogen from dietary protein was obtained with diets containing 8% lactalbumin; nitrogen retention was similar from a diet furnishing the same amount of nitrogen, half as diammonium citrate and half as a mixture of amino acids. Liver protein and fat, and carcass protein were within normal ranges only when the intact dietary protein was increased to the point where the animals were retaining substantial amounts fo nitrogen. Carcass fat of all groups was higher than that found in stock animals of comparable weight. The highest fat (27%) was found in animals fed a diet containing low levels of amino acids, cornstarch, and 50 mg/kg procaine pencillin G.

1650. RELATIONSHIPS OF CLAY AND CORNSTARCH INGESTION TO BIOCHEMICAL FINDINGS IN PREGNANT AND NONPREGNANT WOMEN. Cecile Hoover Edwards, Alta Mae Kemp and Doris Laing (intr. by Pearl P. Swanson). Carver Fndn. and School of Home Economics, Tuskegee Inst., Ala.

Previous studies reported by the Carver Foundation indicate that clay and cornstarch are commonly ingested by rural and suburban women in the southeastern region of the United States. This unusual dietary habit appears to occur as frequently in pregnant as in nonpregnant women, but has not been reported to occur in men. Because of the paucity of information as to the effects of these substances in human subjects, the present study was undertaken. About 30 women who resided in rural areas adjacent to Tuskegee Institute and who attended out-patient clinics of the John A. Andrew Memorial Hospital participated in the study. During the initial interview the subjects were informed of procedures to be followed. On their return visit to the clinic, 24-hr samples of urine, feces and food (including clay and cornstarch), which had been collected the preceding day and fatings blood samples were obtained. Thorough medical examinations were made. The procedure was repeated when the women returned after a period, usually 1 wk, when clay and/or cornstarch had not been ingested. Analyses of hemoglobin, serum protein, calcium and glucose in blood; calcium, phosphate and total solids in urine, nitrogen balances, and calcium, iron and total sha in food and feces will be reported for these two periods. Medical and other biochemical findings will be interpreted in the light of their relationships to the odd dietary practices.

1651. NUTRITION STUDIES WITH DIETS CONTAINING VARYING SOURCES OF AMINO ACID NITROGEN. Gladys A. Emerson and E. E. Howe.* Merck Inst. for Therapeutic Research and Merck Sharp & Dohme Research Labs., Rahway, N. J.

Varying sources of amino acid nitrogen were studied for their effect upon growth and reproduction in the rat. A poor growth response was observed in rats fed amino acid mixtures and an acid digest of casein supplemented with tryptophan as contrasted with a trypsin digest of casein or of casein itself. First-generation rates bred normally. The second-generation offspring of mothers fed the amino acid mixtures weer undersized at weaning. The difference in growth was even more striking at 3 mo. In a 2nd experiment female rats were fed purified diets with protein contents as follows: 24% casein, 34% casein, 24% casein + 10% Armour liver protein and 24% casein + 10% Drackett protein. The percentages of young weaned were 56, 73, 80 and 73, respectively. The weaning weights for the second-generation offspring of mothers fed the liver and Drackett protein-containing rations were 10% above those for the casein groups. The postlactation growth responses for these second-generation animals from lowest to

highest for male rats was in the following order: 24% casein, 34% casein, 24% casein + liver and 24% casein + 10% Drackett protein.

1652. PHENYLALANINE REQUIREMENT OF GROWING CHICKS WITH SPECIAL REFERENCE TO UTILIZATION OF D-ISOMER. Hans Fisher, Dewey Johnson, Jr. and Gilbert Leveille (intr. by M. W. Taylor). Poultry Dept., Rutgers Univ., New Brunswick, N. J.

Using DL-phenylalanine and assuming 100% utilization of the D-isomer, Almquist (J. Nutrition. 34:543, 1947) reported a dietary requirement for the chick of 0.9%. In the present study, the assumption of an equal utilization of both the D- and L-isomers was investigated. In the presence of adequate amounts of tyrosine, the requirement for the L-isomer was found to be approximately 0.4% of the diet and 0.65% of the DL-compound. The D-isomer gave essentially no growth at the 0.5% level and at the 1.0% level supported growth equivalent to 0.27% of the L-isomer. In the absence of tyrosine, 1.0% of L-phenylalanine supported near maximum growth. It is evident, therefore, that the phenylalanine requirement of the growing chick is significantly lower than previously reported. This finding can be explained on the basis of the poor utilization by the chick of the D-isomer of phenylalanine.

1653. ZINK AND ANOTHER INORGANIC GROWTH FACTOR REQUIRED BY TENEBRIO MOLITOR. G. Fraenkel. Dept. of Entomology, Univ. of Illinois, Urbana.

Previous work concerning the vitamin function of carnitine for the beetle, Tenebrio molitor, showed carnitine as the only limiting factor in a diet consisting of casein, glucose, cholesterol, McCollum-Davis Salt mixture and 8 B-vitamins. Subsequently, the responses of the insects to this diet have changed, inasmuch as carnitine no longer is always necessary, but the larvae require for normal growth another addition which is met by the inclusion of 2% water-insoluble yeast fraction (Fraenkel and Leclercq. Arch. Intern. physiol. biochem. 54:601, 1956) or liver powder. The following experiments were performed with whole liver powder (LV) (Viobin Corp.). The active component of LV was extracted in 10% TCA and, after removal of TCA, precipitated with alcohol. Further concentration yielded a powder which after ashing retained its activity. The ash from the original LV, 0.5% of the diet (equivalent to 5-10% LV), was about as active as 2% insoluble yeast, or LV. Of various salts of the following elements tested: Fe, Cu, Mn, Co, Zn, Al, Mo, B, F and I, Zink alone occasionally gave a slight positive growth effect. However, when the water soluble fraction of LV ash was treated with K_2CO_3, neither the precipitate nor filtrate alone promoted growth, while the combined fractions did. Optimal growth resulted from filtrate plus $ZnCl_2$ (0.02% of diet) and zink was absolutely limiting for growth. The nature of the active factor in the carbonate-filtrate is under investigation. The following ions tested so far gave no results: Fe^{+++}, Cu^{++}, Mn^{++}, Co^{++}, Al^{+++}, MoO_4'', BO_3''', F' and I'.

1654. PHYSICAL MEASUREMENTS AND BLOOD FINDINGS OF 185 GIRLS OBSERVED FOR 2-1/2 YEARS. Pilar Garcia,* Charlotte Roderuck and Ercel Eppright. Home Economics Research, Iowa Agricultural Exp. Station, Ames.

One hundred and eighty-five girls, 8-14 years of age, cooperated for 2-1/2 yr in a study of their food habits, physical measurements, concentrations of hemoglobin in blood and concentrations of ascorbic acid, carotenoids, vitamin A and alkaline phosphatase in blood serum. The girls were divided randomly within age and physique groupings for a supplementation program during part of the study. Supplement I added those nutrients most often observed to be unsatisfactory in diets of school children surveyed earlier in Iowa. It contained the equivalent of 1 pint of milk to which vitamin D had been added, one serving of vitamin C-rich foods and one serving of carotene-rich foods. Supplement II was a placebo consisting of a cornstarch pill, fruit juices low in vitamin C and fruits or vegetables containing little or no carotene. Fasting finger-tip blood samples were obtained before and after 14 wk of supplementation in the winter of 1955 and again before and after 21 wk of supplementation during the following fall and winter. The mean hemoglobin concentration for the entire group decreased over 2-1/2 yr, no difference being noted between experimental and control groups. Significant increases in serum carotenoids and ascorbic acid were brought about by supplementation. Seasonal fluctuations were observed in the serum carotenoids and vitamin A values of the control group. No changes occurred in the alkaline phosphatase levels due to supplementation and only minor changes occurred in the physique groupsings throughout the study.

1655. MECHANISM OF TESTOSTERONE-INDUCED N-RETENTION IN RATS. Ernest Geiger and M. E. Nimni.* Depts. of Pharmacology and Biochemistry, School of Medicine, Univ. of Southern California, Los Angeles.

The assumption that the N-retention after testosterone treatment is due to formation of new tissue protein has not yet been satisfactorily proven. The present report is concerned with the effect of TP on the nitrogen balance of rats maintained on rations which normally fail to support protein synthesis. Otherwise complete diets were used employing as the N-source: a) 15% zein, b) 15% gelatin, c) isonitrogenous amino acid mixture of glycine, alanine and Na-glutamate. It was found that the negative N-balance of castrated adult male rats on the zein diets was reversed after the injection of TP (2.5 mg daily for 2-7 days s.c.). Similar results on castrated rats were obtained after TP treatment with gelatin diets. The constant loss of body weight was progressing, however, even during the TP period in spite of positive N-balance and satisfactory total caloric intake. In normal male rats on zein or gelatin diets TP-treatment did not decrease urinary N-excretion. TP did not alter the N-excretion in rats kept on diets containing the amino acid mixture. The results suggest that the TP-induced N-retention may not indicate tissue protein synthesis. It is, however, possible that essential amino acids, normally catabolized in the "wear-and-tear" of body protein are, due to the anabolic effect of TP, reutilized to supplement the incomplete dietary amino acid supply.

1656. EFFECT OF HIGH FAT DIETS ON RIBOFLAVIN REQUIREMENTS OF CATS. S. N. Gershoff* and D. M. Hegsted. Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

Groups of cats have been fed isonitrogenous purified diets containing 26% and 5% fat and varying in riboflavin content for periods up to a year. Among the animals fed diets lacking riboflavin, those fed the high fat diets developed riboflavin deficiency signs and died much sooner than those fed low fat diets. Urinary excretion of riboflavin by cats receiving 2 mg of riboflavin/kg of diet was approximately 25 times greater

in cats receiving low fat than high fat diets. On these regimens cats receiving high fat diets, but not those getting low fat diets, developed signs of chronic riboflavin deficiency. Analyses of urinary and fecal riboflavin at different levels of intake support the premise that high carbohydrate diets exert a sparing effect on riboflavin requirements. More riboflavin can be recovered from the urine and feces than can be accounted for in the diet. This is particularly true when high carbohydrate diets are fed and suggests that the protective effect of high carbohydrate diets is in great part related to increased intestinal synthesis. The amount of riboflavin recovered in the feces of cats showing signs of riboflaving deficiency indicates that much of the fecal riboflavin is not available. The relationship of these observations to the riboflavin requirements of man will be discussed.

1657. METABOLISM OF BIOTIN-DEFICIENT RAT: OXIDATION OF ACETATE-2-C^{14} AND INCORPORATION INTO HEPATIC LIPIDS, CHOLESTEROL AND GLYCOGEN. Mary R. Gram (intr. by Agnes Fay Morgan). Dept. of Home Economics, Univ. of California, Berkeley.

Two series of rats were fed in subgroups as follows: a) biotin-deficient diet, b) adequate control diet, ad lib. and c) control diet in amounts to support weight gains equal to those of their deficient littermates (pair-weighed). The pair-weighed animals rapidly consumed their daily ration and consequently were fasted for 18-20 hr daily. After 6-9 wk on the diet, all rats were fasted for 12 hr, refed for 2 hr and then injected intraperitoneally with sodium acetate-2-C^{14}. The effect of food restriction in the pair-weighed animals was emphasized by the fasting and refeeding procedure proir to injection. By 4 hr after injectionl the pair-weighed animals had oxidized less acetate-2-C^{14} and incorporated more activity into liver lipid and cholesterol than the ad lib. control and deficient animals. Incorporation into liver glycogen was lowest in the pair-weighed animals. Biotin-deficient animals oxidized more acetate than pair-weighed and control animals. Incorporation into liver lipid, total and free cholesterol and glucogen was comparable for the deficient and ad lib. control animals. However, C^{14} was incorporated into carcass lipid equally as well by the deficient as by pair-weighed animals. Therefore, biotin deficiency, per se, does not appear to decrease the rate of fat synthesis. Inasmuch as rate of eating and food restriction were the major factors differentiating ad lib. control and biotin-deficient from the pair-weighed animals, it would appear that these factors have a determining role in the utilization of metabolites by the liver.

1658. HUMAN REQUIREMENT FOR IODINE. Isidor Greenwald. New York Univ. College of Medicine, New York City.

The requirement may be estimated from I) the excretion during fasting; II) the amount required for equilibrium; III) the usual excretion; IV) the amount required to prevent symptoms of deficiency. I) yields about 20 μg; II) 14 to 40 μg; III) (nongoiterogenous areas) 14-894 μg, (goiterogenous areas) 8-1000 μg; IV) the currently accepted values are based on the results of experiments upon rats on certain diets, in no instance of purified constituents. A recent report (J. Argric. Food Chem. 3:72) claimed that goiter was produced in rats on a diet of sunflower-seeds and leaves (grown on a medium free from iodine), yeast and salt whereas normal thyroids were obtained with similar materials grown on 3 similar media containing iodine, but which materials yielded not more than 19, 21 and 38 μg of iodine per 3600 Calories. There is but one properly controlled observation concerning the effect of iodine upon humans (Fed. Proc. 12:423). The prevalence of goiter in school children was greatly reduced by the administration of 5 mg of iodine once a week. However, 16 wk after this was stopped, the goiters were as common as they had been, although the native salt contained an average of 22 parts of iodine per million, 4 times as much as has been credited with reducing the prevalence of goiter in Poland and Switzerland. There is no satisfactory evidence that the basic requirement is not available everywhere. The cause of goiter is to be sought in a biological goitrogen, not in a lack of iodine.

1659. MANIFESTATIONS OF FAT DEFICIENCY IN INFANTS. Arild E. Hansen, Doris J. D. Adam,* Arr Nell Boelsche,* Mary Ellen Haggard,* Hilda F. Wiese, Edward L. Pratt* and Virginia Hunter.* Depts. of Pediatrics, Univ. of Texas School of Medicine, Galveston and Southwestern Med. School, Dallas.

In a study attempting to evaluate the role of fat in infant nutrition, it became apparent that a milk mixture in which fat comprised less than 1.0% of the calories was not well tolerated by very young infants. This milk preparation was made especially for us by a large commercial concern (The Baker Laboratories, Inc.) and provided adequate sources of protein, minerals and vitamins, Carbohydrate was substituted isocalorically for fat so that the diluted milk mixture provided 63 cal/100 ml. Frequency of stools with perianal irritation necessitated changing to a fat containing milk mixture so far in 8 of the 27 babies studied. On 10 occasions distinct changes in the appearance of the skin became discernible within 5-8 wk. The skin felt thicker, was somewhat dry and leathery in texture as compared with other infants receiving at in their diet. A very fine desquamation which in most instances was rather extensive was particularly apparent in the colored infants. The appearance resembled very closely the fine desquamation whcih has been observed over a period of years as an early sign of fat deficiency in puppies. In 2 instances the addition of 2% of the callries as linoleic acid in the form of trilinolein was associated with the complete disappearance of the abnormal skin changes. In another instance, ethyl arachidonate, also fed in amount equivalent to 2% of the calories as arachidonic acid, effected a marked but less dramatic change in the skin. In another infant addition of tripalmitin to equal 2% of the calories as palmitic acid over the same period of time caused no change in the condition of the skin. Shifting to a milk containing fat was associated with disappearance of the abnormal skin symptoms. Study of the serum lipids in infants on the low fat mixture showed extremely low values for 2 and 4 double bond fatty acids and high values for the 3 double bond fatty acids. These preliminary findings suggest that the young human infant may show clinical signs of fat deficiency.

1660. EFFECT OF TYPE OF CARBOHYDRATE ON PROTEIN UTILIZATION. A. E. Harper and H. E. Spivey.* Dept. of Biochemistry, Univ. of Wisconsin, Madison.

The basis for the superiority of dextrin over sucrose as the carbohydrate in low-protein diets for young rats remains obscure. Rate of gain, food intake and efficiency of food utilization of young rats consuming diets low in protein (Harper and Katayama. J. Nutrition 49:251, 1953), and nitrogen retention of mature rats receiving low levels of amino acids (Womack and

Marshall. J. Nutrition 52:51, 1954), are greater when "dextrin" is substituted for sucrose in these diets. Dextrin does not appreciably improve growth when young rats receiving this crotohydrate are pair-fed with those given sucrose; therefore, improved nitrogen retention can account for only part of the beneficial effect of dextrin. To test the hypothesis that differences in food intake were caused by differences in the osmotic effects of these carbohydrates, food and water sonsumption were measured during spaced-feeding experiments. Growth and food intake of rats receiving the dextrin type diet were greater but the lower food intake of those receiving sucrose was not associated with increased water consumption. However, when rats were killed at intervals after consuming a test meal containing 10% of casein and about 80% of carbohydrate, the volume of the stomach contents of rats receiving sucrose was greater up to 5 hr after eating because substantially more water was present in the stomach. Further experiments are under way to determine whether the greater retention of fluid in the stomach of rats receiving sucrose causes reduced food ingestion and, indirectly, a slower rate of growth.

1661. REACTION MECHANISM AND SUBSTRATE SPECIFICITY OF A FUNGAL CELLULASE SYSTEM. John H. Hash and Kendall W. King (intr. ty A. E. Schaefer). Dept. of Biochemistry and Nutrition, Virginia Agricultural Exp. Station, Blacksburg.

The cellulolytic enzymes in culture filtrates of Myrothecium verrucaria have been partially fractionated by elution from a column of alakli-swollen cellulose using buffers of increasing pH and ion strength. Effluent fractions were assayed for their capacity to hydrolyze carboxymethyl celluloses of 2 degrees of polymerization, water-insoluble cellulose dextrins, water-soluble cellulose dextrins, cellobiose, 6-β bromo-2-0-naphthyl-β-D-glucopyranoside and 4-0--D-glucopyranosyl-D-glucitol. Production of glucose during hydrolysis of cellulose dextrins by fractions having no cellobiase indicates that cellobiose is not an obligatory glucose precursor. Fractions containing active cellobiase also exhibit glucotransferase activity. Cellulase fractions which are void of transferase activity accumulate glucose, cellobiose and higher saccharides when hydrolyzing water-insoluble dextirns indicating that water-soluble dextrins are true intermediates of hydrolysis rather than reversion products. The separations show clearly that β-aryl-glucosidase and cellobiase activities cannot be attributed to the same enzyme. All of the data are consistent with the hypothesis of random cleavages of glycosidic linkages in cellulose by a variety of enzymes having difference specificities with regard to substrate chain length.

1662. FACTORS INFLUENCING ABSORBABILITY OF SATURATED FATS. David C. Herting,* Stanley R. Ames* and Philip L. Harris. Research Labs., Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y.

Absorbabilities were determined for various atsurated fats including fully hydrogenated lard (Iodine No. 0.1) and monoglycerides fatty acids or acetylated monoglycerides derived from hydrogenated lard. The low utilization of hydrogenated lard by the rat when fed alone was doubled by incorporating lard into the diet. Similar results were observed after adding lard to diets containing saturated monoglycerides or fatty acids. Feeding diets containing mixtures of saturated and unsaturated acetylated monoglycerides at levels up to 50% or diets containing 25 or 50% of saturated acetylated monoglycerides with additional natural fat resulted in increased absorbability of the saturated fat in all groups. When mixtures of saturated and unsaturated fats were fed, the absorbabilities of the saturated fats were calculated by assuming essentially complete absorption of the unsaturated fat in accordance with the finding that the iodine numbers of the fecal fatty acids were very low. The absorbability of saturated acetylated monoglycerides when fed alone decreased as the level of fat in the diet was increased from 5 to 50%. At low levels of dietary fat, the saturated fatty acids from the acetylated monoglycerides were absorbed much more than were those from fully hydrogenated lard, indicating a beneficial effect of the acetyl groups on utilization of the long-chain fatty acids. In order to determine accurately the fecal fat after feeding completely saturated fat, revised analytical procedures were necessary. The absorbability of a fat was defined as the difference between consumption and excretion of its constituent fatty acids expressed as a percentage of fatty acid consumed.

1663. IN VIVO AND IN VITRO HYDROLYSIS OF VITAMIN A ESTERS AND ESTERIFICATION OF VITAMIN A ALCOHOL. Edward G. High, J. Ronald Powell* and Henry B. Bright.* Dept. of Biochemistry, Meharry Med. College, Nashville, Tenn.

The previous study of the in vitro hydrolysis and esterification if vitamin A (Fed. Proc. 15:1810, 1956) has been extended. In the in vivo studies vitamin A-deficient rats were injected via tail vein or intracardially with an aqueous dispersion of the vitamin A prepared with Tween 80. At various time intervals, rats were killed and the liver and kidneys were analyzed for vitamin A alcohol and ester. Between 5 and 30 min after the injection of vitamin A, alcohol, approximately an equal distribution of ester and alcohol, was found in the kidneys while in the liver the alcohol predominated. Between 2 and 48 hr, liver vitamin A ester increased. Five minutes after injecting vitamin A acetate, appreciable hydrolysis of the ester had occurred; in 10 min after the injection the alcohol predominated in the liver and in 30 min the ester again predominated. In the case of the injected vitamin A palmitate, hydrolysis proceeded but at a much slower rate. In an attempt to localize the site of esterification of vitamin A alcohol, vitamin A-deficient rats were either nephrectomized, enterectomized, or both and injected with vitamin A alcohol, Although the nephrectomized rats exhibited a slower rate of esterification of vitamin A alcohol as compared to normal rats, esterification of vitamin A alcohol still proceeded in all the experimental groups. Vitamin A acetate, caprylate, laurate and palmitate were hydrolyzed by liver homogenates with the palmitate exhibiting the least, the laurate next to the least and the acetate the greatest rate of hydrolysis. These results suggest that the rate of hydrolysis of vitamin A esters by tissue homogenates is a function of the chain length of the ester. (Supported in part by a research grant from the Natl. Science Fndn.)

1664. LEVELS OF MENADIONE AND RESISTANCE TO FOWL TYPHOID. C. H. Hill and H. W. Garren (intr. by G. H. Wise). Dept. of Poultry Science, North Carolina State College, Raleigh.

Previous work from this laboratory (Ann. New York Acad. Sci. 1955) indicated that the resistance of chicks to fowl typhoid could be increased by raising the dietary vitamin levels. The results of this work indicated that the menadione requirement for maximum

resistance to fowl typhoid exceeding 10 mg/lb. This level far exceeds the N.R.C. suggested requirement for growth (0.18 mg/lb). Therefore, further studies have been conducted to determine the most effective level of menadione and menadione sodium bisulfite (MSB), in combination with high levels of the other essential vitamins. In these studies a corn-soybean meal basal was used which contained adequate amounts of all the vitamins essential for growth. The mandione or MSB level of the basal diet was 10 mg/lb. After 4 wk on the various treatments the birds were inoculated with a culture of Salmonella gallinarum and mortality was observed. In the first experiment 40 birds were used per treatment. Eight groups of birds were fed the basal diet with menadione, a diet containing high levels of all the required vitamins including ascorbic acid and menadione at 10 mg/lb, a high vitamin diet with menadione at 25 mg/lb, and a high vitamin diet with menadione at 50 mg/lb. The percentage survivors on these diets were 30.5, 44.4, 59.5 and 41.2, respectively. When vitamin K was supplied as MSB, the corresponding percentage survivors were 25.0, 58.8, 42.1 and 34.2, respectively. However, in a 2nd experiment using 80 birds per treatment, increased resistance was not noted until the level of vitamin K reached 50 mg/lb with either menadione or MSB. There was no effect of either vitamin K supplement on the prothrombin times during the course of the disease.

1665. INFLUENCE OF TORULA YEAST AND BREWERS' DRIED YEAST ON REPROCUCTION IN CHICKENS AND LIVABILITY OF CHICKS. Leo S. Jensen,* Ramon E. Fry* and James McGinnis. State College of Washington, Pullman.

In a preliminary study with White Leghorn hens it was found that a marked decrease in egg production occurred when they were placed on diets containing Torula yeast as the source of protein. Hatchability of fertile eggs decreased also except when supplementary alpha-tocopheryl acetate was added. It was observed that many of the chicks hatched from the diets not supplemented with vitamin E died in the incubator after emerging from the shells. Since the number of eggs produced in the preliminary experiment was small because of the poor production, a 2nd study was conducted. The change from standard diet to experimental diets was made gradually to reduce the adverse effects on rate of production. In this experiment, egg production was satisfactory but there was a prompt decline in hatchability of fertile eggs from all treatments except the vitamin E supplementation and the standard diet. All hatched chicks from all treatments were placed on a standard chick diet and observed 4 wk for mortality. It was found that approximately 45% of the chicks hatched from the Torula yeast diet died and that vitamin E supplementation of the hen diet prevented this mortality. Brewers' dried yeast in the hen diet prevented the high chick mortality even though it did not support high hatchability or chick livability. The results indicate that both Torula yeast and brewers' dried yeast are deficient in a hatchability factor which is either vitamin E or a factor which vitamin E can replace.

1666. EFFECTS OF DIETARY CARBOHYDRATES ON UTILIZATION OF BIOTIN IN RATS. Dorothy R. Jutton* and Helen T. Parsons. School of Home Economics, Univ. of Wisconsin, Madison.

Protection from biotin depletion in rats fed a raw egg white ration containing cooked cornstarch was indicated by better growth, higher biotin levels in livers and less severe deficiency manifestations as compared with rats fed a similar ration containing sucrose. On the average, growth rates of the groups fed cooked cornstarch were 47% to 285% greater than those fed the sucrose ration. The biotin concentration in the livers of the former group were 2 to 3 times as great as the latter group. The evidence of increased biotin synthesis in the superior group was equivocal, since the average daily elimination of biotin in the feces of these rates was within the same range as the amounts eliminated by the rats fed the sucrose ration. From the balance data of these 2 groups, it appeared that the form of carbohydrate in the ration might have had an influence on the stability of the avidin-biotin complex present in the raw egg white of the ingested ration or that formed from combination with synthesized biotin in the intestinal tract. Differences in urinary biotin excretion were minimal as compared with the differences in liver biotin levels but a trend toward higher urinary biotin for the superior group was observed.

1667. EFFECT OF DIETARY PROTEIN AND FAT ON CHANGES OF SERUM CHOLESTEROL IN MATURE ROOSTERS. M. Kokatnur,* N. T. Rand,* F. A. Kummerow* and H. M. Scott. Depts. of Food Technology and Animal Science, Univ. of Illinois, Urbana.

Seventy-two mature roosters were divided into 2 groups, one group was fed a basal ration and the other the basal ration plus 1% cholesterol for 10 days in order to induce hypercholesteremia. The birds were then divided into 12 groups of 6 birds each and fed for 3 wk diets containing 7.5, 15 or 30% protein and 0 or 15% corn oil. Samples of blood were obtained from each bird via heart puncture at the beginning, the 10th and the 21st day of the experimental period and the serum cholesterol level of each sample determined by the Sperry-Schoenheimer method. All data were subjected to statistical analysis. The results indicated that the serum cholesterol level was significantly higher at the 7.5% protein level. The serum cholesterol levels of all of the hypercholesteremic birds dropped rapidly during the 3-wk experimental period. However, it did not drop to normal values in this period of time unless the protein level was high. The level of dietary fat had significance when the level of dietary protein was inadequate but not when protein was adequate.

1668. OCCURRENCE OF ZINC IN MOSQUITO AND OTHER INSECTS. Calvin A. Lang (intr. by Ernestine B. McCollum). Div. of Med. Entomology, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.

High concentrations of zinc have been found in certain sex organs and their secretions of man, laboratory animals and the silk worm as compared to low amounts found in the other soft tissues of these animals. These observations together with the fact that mosquitoes and other insects exhibit high reproductive potentials suggested an examination of the zinc content of these invertebrates. Samples of 7 insect species (Aedes aegypti, Culex pipiens, Culex molestus, Tribolium confusum, Oncopeltus fasciatus, Lymantria dispar and Apis mellifera) representing 5 biological orders were collected from laboratory reared colonies, and spectrographic analyses, using a spark excitation method and microchemical determinations, using the method of Vallee and Gibson, were performed on the whole carcasses. The zinc content of most species was 0.05-0.2 μg/mg dry weight, an amount similar to that reported for many mammalian tissues. However, the concentration

present in the various mosquito species ranged from 0.6-1.2 μg/mg, an amount 5-10 times greater than that found in the other insects. Furthermore, this trace element appeared to be confined exclusively to the abdomen thereby raising the concentration to about 1.8 μg/mg. Other data indicate that the mosquito larvae are able to accumulate zinc from media containing relatively low levels of zinc. (Supported in part by a research grant from the Public Health Service.)

1669. CHANGES WITH AGE IN BASAL METABOLIC RATE OF ADULT WOMEN. Ruth Leverton, Alice Biester,* Isabel Pesek,* Lida Burrill,* Margaret Mangel* and Dena C. Cederquist. Univ. of Nebraska, Lincoln, Univ. of Minnesota, Minneapolis, Iowa State College, Ames, South Dakota State College, Brookings, Univ. of Missouri, Columbia and Michigan State Univ., East Lansing.

Measurement of the basal metabolic rate, based on oxygen consumption of an individual under standard conditions, may prove to be a useful tool for assessing the performance of actively functioning tissue in the body. One physiological aspect of aging appears to be a reduction in the number and/or the rate of metabolizing units in the organism. An indication of aging thus might be reflected in decreased oxygen consumption. A total of 620 women, 30-89 years of age, have been studied in the North Central States. Basal oxygen consumption (expressed as calories per square meter per hour) decreased with each decade from 30-59 yr. An increase in oxygen consumption in the 7th decade was followed by a decrease in the subsequent decade. A possible explanation for the observed data will be offered.

1670. AVAILABILITY OF VALINE FOR HUMAN SUBJECTS. Hellen Linkswiler, Hazel Metz Fox and Donna Geschwender (intr. by May S. Reynolds). Dept. of Home Economics, Univ. of Nebraska, Lincoln.

The availability of valine from corn has been determined in young women. Twelve college students served as subjects. A semisynthetic diet was used which consisted of fat, cornstarch, minerals, vitamins and fruits low in nitrogen. The 8 essential amino acids were fed at approximately twice the levels which have been suggested as the minimum daily requirements for young women. Arginine, histidine, cystine and tyrosine were also given. Glycine, diammonium citrate and glutamic acid were added to bring the total nitrogen intake to 10 gm daily. Periods during which the semisynthetic diet was given were alternated with periods in which corn was added to the purified diet and the crystalline amino acids were reduced by the amount supplied by the corn. During the periods when availability from corn was tested, no synthetic valine was given. The daily valine intake was decreased stepwise from 600 to 275 mg. Even at the lowest level of intake, a distinctly negative nitrogen balance was observed in only one subject. Using nitrogen balance as the criterion of comparison, valine from corn was as well utilized as synthetic valine.

1671. NEW METHOD OF FRACTIONATING CORN PROTEINS AND ITS USE IN ASSAY OF DIFFERENT CORN VARIETIES. Norman E. Lloyd,* Ricardo Bressani* and Edwin T. Mertz. Dept. of Biochemistry, Purdue Univ., Lafayette, Ind.

The grains of several U.S. and Guatemalan varieties of corn (Zea mays) were separated into seed coat, germ and endosperm. The proteins of the germs and endosperms were extracted with an alkaline copper reagent (Mertz and Bressani. Cereal Chem. 34: 1957) and the extracts fractionated as follows: The corn protein extract (pH 11-12) is adjusted to pH 3.0 and centrifuged. The supernatant is the acid soluble fraction. The precipitate is dissolved in 1 part of dilute NaOH sol. (pH 11-12), 2 parts of abs. ethanol added, the pH brought to 6.0 and the mixture centrifuged. The supernatant is the alkali-alcohol-soluble fraction and the precipitate, the alkali-soluble fraction. In the endosperm samples, the acid-soluble proteins varied from 17 to 26%, the alkali-alcohol-soluble fraction (zein) from 41 to 60% and the alkali-soluble fraction (glutelins) from 17 to 31% of the total nitrogen. Similar treatment of the corn germs gave 30-40% acid-soluble, 5-10% alkali-alcohol-soluble and 49-54% alkali-soluble as percentage of total nitrogen. The pooled acid-soluble proteins of the endosperms contained 2.90% lysine, the pooled zein 0.08% and the pooled glutelins 2.16% (16% nitrogen basis). In contrast, the pooled acid-soluble proteins of the germs contained 6.4% lysine, the pooled alkali-alcohol soluble proteins 1.0% and the pooled alkali-soluble proteins 5.8%. The new fractionation method should be helpful to the genetricist in breeding for corn proteins of higher biological value.

1672. A COMPARATIVE NUTRITION STUDY. Thomas D. Luckey. Dept. of Biochemistry, School of Medicine, Univ. of Missouri, Columbia.

This progress report presents some of the work done with a synthetic-type diet (Science 120:396, 1954) to which two minor changes have been made for the later work: one-half of the fiber was changed to cellophane spangles to prevent impaction in ruminants and molybdenum (0.03% $Na_2MoO_4.2H_2O$) was added. Then 10 transfers under sterile conditions were made for each species of mircoorganisms. A 10-fold increase in body weight is considered significant as a criterion of success of the diet with each macro-species. Reproduction was obtained with cockroaches (4 generations), snails (2 generations), chickens, guinea pigs and mice (4 generations). Representative of the following phyla or major divisions have been reared satisfactorily: Schizophyta (Escherichia coli, Lactobacillus arabinosis and Streptococcus faecalis), Myxomycophata (Physarum polyecphalum was maintained for short periods of time), Eumycophata (Penicillium expansum and Saccharomyces Cerevisiae), Algae (Chlorella sp), Tracheophyta (Lycopersicon esulentum, the tomato) Protozoa (Tetrahymena gelleii), Mollusca (the snail, Helisoma companulata), Arthropoda (Blattella germanica) and Chordata (a white strain of Mus musculus). It also seemed desirable to rear one example of each class of vertebrates in the phylum chordata. In Osteichthyes gold fish (Carassius auratus) and guppies (Cambusia affinis) grew satisfactorily. In Amphibia, tadpoles of the frog (Rana pipiens) grew slowly and about 1 out of 10 metamorphosed satisfactorily. In Reptilia, one turtle (apparently Clemmys guttata) ate the diet every day until it got lost; other turtles did not eat well. In the class Aves, chicks (White Leghorn variety of Gallus domesticus) Peking ducks and turkeys (Meleagris galloparo) grew well when fed this diet; the chicks were kept until they laid eggs (and one of thees hatched). In the class Mammalia, one example of most Orders are being tried: success has been obtained with Marsupialia (Opossums); Carnivora (cats and dogs); Primates (monkeys); Rodentia (rats, mice, guinea pigs); Lagomorpha (rabbits); and Artiodacytla (pig). Other representatives are being tried to obtain further generalization of the thesis that many diverse species can be reared on a standard synthetic-type diet.

1673. QUANTITATIVE UTILIZATION OF TRYPTOPHAN FROM FOODS BY THE RAT. C. H. Lushbough (intr. by B. S. Schweigert). American Meat Inst. Fndn., Univ. of Chicago, Chicago, Illinois.

This study was undertaken to determine how much of the tryptophan present in several foods is used to support growth in the male weanling rat. The basal ration, designed to be adequate in all known required nutrients except tryptophan, was based on 10% oxidized casein + 4% untreated casein + supplements of cystine, methionine and tyrosine. It provided 38 mg tryptophan and 2 mg niacin/100 gm. The growth response of animals receiving greaded supplements of L-tryptophan was compared with that obtained when fresh or cooked meats, cereals, legumes or skim milk were fed to provide limiting levels of tryptophan supplementation. The foods tested were added to provide 20-80 mg tryptophan/100 gm ration in the utilization tests. Tryptophan content of the foods studied was assayed both microbiologically and chemically and the results were in good agreement. Tryptophan utilization results were: meats, 93%-107%; rolled oats, unheated and normally processed soybean meals, 117%-132%; overheated soybean meal, nonfat dry milk, and split peas, 75%-85%. Fecal tryptophan determinations for L- and DL-tryptophan and the foods indicated 90%-99% digestibility of ingested tryptophan. The tryptophan utilization values above 100% may hvae resulted from low tryptophan assay values for the foods; or tryptophan may be more efficiently utilized from these food proteins than when provided as L-tryptophan supplements. Amino acid utilization values previously reported for several foods ranged from 49% to 98% for lysine and 48% to 83% for methionine. The high values observed for tryptophan utilization (75-132%) gain added significance when compared to the lysine and methionine utilization values.

1674. ENZYMATIC DETERMINATION OF POLYUNSATURATED FATTY ACIDS. J. MacGee* and F. H. Mattson. Research Div., Procter and Gamble Co., Cincinnati, Ohio.

A simple and rapid enzymatic method for the determination of linoleic, linolenic and arachidonic acids has been developed. The potassium salts of the fatty acids are oxidized by atmospheric oxygen in the presence of the enzyme lipoxidase and the absorption of the conjugated diene hydroperoxides is measured at 234 mμ. This method has the advantage over the chemical isomerization technique that only compounds having a methylene-interrupted diene structure in which the double bonds are in the cis-configuration can act as substrates. As little as 4 γ of linoleic acid can be determined. The method will be described and its application to a variety of fats and pure fatty acids will be given.

1675. OBESITY IN A LONG-TERM PROGRAM. Ruth G. MacIntosh,* Dorothy Daley,* D. N. R. Jackson* and Barbara A. McLaren. Univ. of Toronto and Women's College Hosp., Toronto, Canada.

Effects of various factors in weight reduction on 4 groups of obese women from 16-65 years of age and ranging in weight from 150 to 300 lb, were studied in a 14-momth program. The 4 groups received different treatment: I) dietary restriction, physical exercise, appetite depressants plus lipotropic factors; II) dietary restriction plus lipotropic factors only; III) dietary restriction, physical exercise plus placebos; IV) control group - dietary restriction only. Evaluation of previous eating patterns in relation to recommended daily allowances and an estimation of the patients' probable caloric requirements by means of the Boothby and Bergson Food Nomogram were made at the beginning of the study. Group instruction in nutritional principles plus individual checks and advice on dietary problems were given weekly. Simple exercises to counteract faulty posture and muscular weakness were introduced when indicated in the experimental plan. Patients were given individual medical examination at the beginning and periodically throughout the study to determine the apparent effect of treatment. Weights were recorded each week and changes in body measurements were observed at intervals throughout the program. The prescribed flexibility exercises were checked weekly. Clinical tests included: 1) hemoglobin and 2) urinalysis—at 6-wk intervals; 3) liver function tests - a) cephalin cholesterol flocculation, b) mercuric chloride and c) thymol turbidity; 4) serum proteins with electrophoresis; 5) basal metabolic rate; 6) serum cholesterol; 7) fasting blood sugar.

1676. ABSORPTION AND DISTRIBUTION OF Cr^{51} IN THE ALBINO RAT. Robert D. Mackenzie* and C. A. Hoppert. Kedzie Chemical Lab., Michigan State Univ., E. Lansing.

When trivalent Cr^{51} is fed orally, most of it is excreted in the feces within 4 days and only about 0.5% is absorbed (Visek. et al. Proc. Soc. Exper. Biol. & Med. 84:610, 1953). Since hexavalent Cr^{51} was not given orally in the above study, it was fed by stomach tube to albino rats. The activity of the stomach, intestine, liver, kidney, urine, feces and blood was determined at intervals of 6, 24, 72 and 168 hr. Total recoveries varied between 88-97.5% of the injected dose. Hexavalent Cr^{51} was absorbed to a greater extent than trivalent. When rats were starved for 24 hr absorption was appreciably greater than when fed ad lib. Hexavalent Cr is absorbed by the red blood cell whereas trivalent is not (Gray and Sterling J. Clin. Invest. 29:1604, 1950). A measurement of the activity of blood plasma and red cells would therefore reflect the valence state of the absorbed Cr. Accordingly 2 experiments were done, taking blood samples 3-4 hr after injection and determining the activity of the plasma and red cells. In the 1st experiment hexavalent and trivalent Cr^{51} were given by stomach tube to groups of fasted and nonfasted animals. In the 2nd, Cr^{3+}, Cr^{6+} and a mixture of equal volumes of both were injected directly tino the small intestine about 1.5 in below the stomach. In general, the results indicate that hexavalent Cr is appreciably reduced in the stomach and to a greater extent when food is present; also hexavalent Cr is absorbed from the intestine to a much larger extent than trivalent.

1677. NON-ENZYMIC DECARBOXYLATION OF PYRUVATE BY THIAMINE AND THIAMINE ANALOGS. Emeteria Y. Manzo and David E. Metzler (intr. by Byron H. Thomas). Dept. of Chemistry, Iowa State College, Ames.

The nonenzymic thiamine-catalyzed decarboxylation of pyruvate (Mizuhara, Tamura and Arata. Proc. Japan Acad. 27:302, 1951) provides a model system whose further study should assist in understanding the biological function of thiamine. We have verified the results of Mizuhara et al. and have attempted to find the optimum conditions for study of the reaction and to learn the structural requirements for the catalyst. Acetoin formation, carbon dioxide evolution and pyruvate and thiamine loses have been measured following the incubation of thiamine and pyruvate anaerobically at 50°. Acetoin is the principal product; no acetaldehyde is detected. Addition of acetaldehyde does not increase the rate of reaction but acetoin is produced

very slowly when acetaldehyde alone is incubrated with thiamine. Conditions have been devised under which each thiamine molecule, on the average, will cause the decarboxylation of 1 molecule of pyruvate in 1 hr and of 6 molecules of pyruvate in 22 hr at 50°. The optimal pH in borate buffers is 8.6-9. The catalytic effects of a variety of thiamine analogs have been compared in the model system. In general the results confirm those independently obtained by Breslow (Chem. and Ind. B.I.F. Review, R28, 1956). Some approximate activities relative to thiamine as 100 are: acetylthiamine, 120; 3, 4-dimethyl-5-(2-hydroxyethyl) thiazolium chloride, 2; 3-benzyl-4-methyl-5-(2-hydroxyethyl) thiazolium chloride, 40; 3-(o-nitrobenzyl)-4-methyl-5-(2-hydroxyethyl) thiazolium chloride, 4; neopyrithiamine, 0.

1678. PURIFIED DIETS FOR RUMINANTS. Gennard Matrone,* H. A. Ramsey* and G. H. Wise. Nutrition Section, North Carolina State College, Raleigh.

The efficacy of 3 purified diets, each containing a different mixture of energy-yielding constituents, for growth of lambs was investigated. Preliminary results show that lambs fed a diet containing glucose and 31.9% of the salts of the volatile acids, acetic, propionic and cutyric, grew better than lambs on diets containing either starch and glucose or starch, glucose and cellulose. In contrast with these results, mice fed the diet containing glucose and volatile acids died within a period of 3 wk whereas mice fed the diet containing glucose and starch grew normally.

1679. PRODUCTION OF OSTEOMALACIA AND SENILE OSTEOPOROSIS. J. F. McClendon and J. Gershon-Cohen,* Albert Einstein Med. Ctr., Northern Div., Philadelphia, Pa.

Since there are about 1200 gm of calcium in the adult human skeleton and osteoporosis is noted in x-ray ordinarily when 40% is depleted, a negative calcium balance of 100 mg/day would take 13 yea to produce senile osteoporosis. In rat experiments, 40% of the calcium of the skeleton was depleted by a diet devoid of vitamin D but containing 0.025% calcium, during a comparable fraction of the life span. Therefore senile osteoporosis may be nutritional in origin, although changes in sex hormone production and muscular activity may be contributing factors. No one has accureately determined calcium intake in humans over so long a period. A diet containing 0.1% phosphorus, half of which was in phytin and therefore considered nonavailable, produced a mild form of osteomalacia in rats. But the bones stopped growing in length and therefore the hormone balance may have been disturbed. The calcium and ash was depleted more than the protein and therefore the percentage of protein (collagen) was increased. (This differs from osteoporosis, in which the ash and protein are depleted at the same rate and the percentage of protein is normal.) No urinary calculi were produced but our diet contained no citrate. Urinary calculi have been reported by others on diets still lower in phosphorus and some containing citrate in an Osborne-Mendel type of salt mixture devoid of phosphorus. This work has been delayed 5 yr due to difficulties in determining bone density accurately.

1680. PORPHYRINS OF TUMOR TISSUE. Irving Melcer,* John Piligian* and James M. Orten. Dept. of Physiological Chemistry, Wayne State Univ. College of Medicine, Detroit, Mich.

Earlier investigations have indicated the presence of significant amounts of porphyrins in certain types of tumor tissue (chloroma, female genital tract, etc.) and increased amounts of prophyrins in the Harderian glands of cancer-susceptible strains of mice. Also, injected prophyrins appear to localize in tumor tissues. Studies in this laboratory to date have not demonstrated any significant prophyrin concentration with the possible exception of a uro-type porphyrin in 3 types of tumor tissue (transplanted LC-18 and transplanted Walker carcinoma tissue in rats and human lung carcinoma tissue). It is possible, however, that porphyrin synthesis may occur in tumor tissue in excess of that found in other tissues. In order to investigate this possibility, homogenates of rat tumor tissue (transplanted LC-18 and Walker carcinoma) were incubated with delta-amino-levulinic acid as a precursor and prophyrin biosynthesis was compared with that in nucleated erythrocytes and liver tissue as controls. The porphyrins formed were extracted, converted to the methyl esters, chromatographed on silica gel and the amounts formed determined in a Beckman B photometer. The results of the study now in progress will be discussed. (Aided by grant No. C-2144, Natl. Insts. of Health, PHS.)

1681. EFFECTS OF VARIOUS PLASMA FRACTIONS ON ELECTROPHORETIC MOBILITY OF EMULSIFIED NEUTRAL FAT. H. C. Meng,* James A. Young,* Harrison H. Shoulders, Jr.* and John B. Youmans. Depts. of Physiology and Medicine, Vanderbilt Univ. Med. School, Nashville, Tenn.

The mobility of emulsified neutral fat particles was measured by cataphoresis. The average mobility of emulsion particles ranging from 0.5 to 4 μ in diameter in 0.15 M phosphate buffer at pH 7.0 was 3.48 μ/sec/volt/cm. Preincubation of fat particles with serum reduced the mobility, the degree of reduction depending on the serum concentration. Preincubation of fat particles with serum albumin reduced their mobility only when relatively high concentration of the protein was present. On the other hand, the mobility was considerably reduced by relatively low concentration of serum globulins. For example, with a fat concentration of 0.02% the mobilities were 1.40, 2.76 and 1.34 μ/sec/volt/cm in 0.05% whole serum, 0.03% serum albumin and 0.01% globulin, respectively. The mobility of fat particles in serum obtained following the i.v. administration of triglyceride emulsion was 1.55 μ/sec/volt/cm. The mobilities of chylomicra of lipemic plasma and thoracic duct lymph were 1.27 and 1.58 μ/sec/volt/cm, respectively. It is concluded that 1) when triglyceride emulsion is incubated with plasma the fat particles become coated with protein and the protein-coated fat particles migrate at a considerably reduced rate; 2) serum globulins seem to have a higher affinity than albumin for the particles and 3) it appears that the i.v. administered fat particles become coated with proteins as physiologically occurring chylomicra.

1682. UNIDENTIFIED GROWTH FACTOR IN EGG YOLK. Henry Menge,* Robert J. Lillie and C. A. Denton, U.S. Dept. of Agriculture, Peltsville, Md.

It has been reported (Denton et al. Fed. Proc. 13: 455, 1954) that egg yolk (fresh or dried) added to a corn-soya type ration stimulates growth of chicks to 4 wk as much as 18% above that of the basal group. These studies have initiated further research designed to investigate some of the physical and chemical properties of the unidentified growth factor present in yolk. Preliminary fractionation procedures have shown that the active principle is fat soluble and can be extracted from dried yolk using the common fat solvents. The nonphospholipid and the nonsaponifiable portions of an ethanol extract of yolk contain the growth factor.

Oleic and linoleic acids and lecithin were inactive. The factor is heat labile and loss of activity was observed after ultraviolet irradiation. The ash of yolk gave no growth response. Adjustments in the caloric level of the diet and omission of alfalfa (saponins) from the ration did not affect the growth response to yolk. Yolk exhibits a growth response even though the diet is supplemented with fish solubles and lard. It is apparent that yolk contains an unidentified chick growth factor not identical with the fish factor. These results do not support the suggestion of Hooper et al. (Poultry Sci. 35:195, 1956) that egg yolk owes its major growth promoting ability to the fish factor contained therein.

1683. EFFECT OF INTRINSIC FACTOR ON UPTAKE OF RADIOACTIVE VITAMIN B_{12} BY SLICES OF RAT LIVER. O. Neal Miller, J. L. Raney* and Fred M. Hunter.* Dept. of Biochemistry and Lab. of Nutrition and Metabolism, Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La.

Slices were prepared, from chilled liver of rats that had been fed a diet deficient in vitamin B_{12} and containing 0.04-0.05% iodinated casein and incubated (37°) in Warburg vessels containing buffer (Hastings et al. J. Biol. Chem. 194:69, 1952) pyruvate and 29.2 mμg of radioactive B_{12} (75 μc/μm). The gas phase was 5% CO_2 and 95% O_2. At the end of incubation, slices were washed (10 sec) in buffer, transferred to glass cuvettes and assayed in a scintillation well-type counter. The uptake of vitamin B_{12}, corrected for controls and weight of tissue, increased with time of incubation and concentration of vitamin B_{12} in the media. One hundred μg/flask of intrinsic factor (IF) (Hog mucosa concentrate, biologically active at 5 mg/day) stimulated uptake by 60%, while larger amounts inhibited. As little as 5 μg of this preparation stimulated uptake, Boiled IF or lysozyme stimulated only 13%. Pink dobalamin protein, pepsin or serum caused no stimulation, while crude gelatin stimulated slightly. Slices of livers from animals receiving a diet enriched with B_{12} showed similar ability to absorb vitamin B_{12} and was equally stimulated by IF. Slices of liver from rats receiving a diet devoid of iodinated casein showed a reduced ability to absorb vitamin B_{12} but stimulation by IF was as good or better regardless of whether or not the diet contained added vitamin. These data suggest that IF may aid absorption of vitamin B_{12} at the cullular level other than intestinal mucosa.

1684. DIETARY INTAKE AND TISSUE LEVELS OF ASCORBIC ACID, FOLIC ACID AND CITROVORUM FACTOR IN THE MONKEY. Krishna P. Misra,* Calvin W. Woodruff and William J. Darby. Div. of Nutrition, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

Thirty-eight (33 mucaca mulatta and 5 cymanolagous) monkeys of both sexes, average weight 2.5 kg, were fed a purified sucrose-casein diet (Dutra de Oliveira et al. J. Nutrition 59:515, 1956). Thedaily intake of ascorbic and folic acids was varied, respectively, over the ranges of 0-50 mg and 0-200 μg in various combinations. The monkeys were killed in extremis and livers assayed for ascorbic acid (dinitrophenylhydrazine) folic acid and citrovorum factor (microbiological). The mean ascorbic acid content of livers of monkeys fed 0, 1, 3 and 50 mg ascorbic acid was 2.06±0.32; 2.35±0.45; 4.68±0.47 and 13.27± 0.36 mg/100 gm, respectively. These levels were not influenced by variations in dietary intake of folic acid. Folic acid content of livers on 0, 10 and 200 mcg folic acid intake averaged 419±98; 235±34 and 2258±218 mμg/gm liver, respectively. The citrovorum factor content of these livers averaged 192±12; 208±63 and 929±290 mμg/gm liver, respectively. Higher intakes of ascorbic acid were associated with greater concentrations of citrovorum factor in the liver at each intake of folic acid. Thus, on an intake of 200 μg folic acid the liver citrovorum content increased from 568±141 to 2375±26 mμg/gm with the increase in dietary ascorbic from 0 to 50 mg. The citrovorum factor content of the liver of the monkey reflects both the ascorbic and folic acid intake.

1685. DIPHOSPHOPYRIDINE NUCLEOTIDE CONTENT OF WHOLE BLOOD AND BLOOD FRACTIONS OF WOMEN ON CONTROLLED DIET. Nina H. Morley* and Clara A. Storvick. Nutrition Research Lab., Agricultural Exp. Station, Oregon State College, Corvallis.

Daily DPN levels of the whole blood and blood fractions of 4 women receiving for 30 days a controlled diet containing 8 mg niacin and 750 mg tryptophan were determined microfluorometrically. For the whole period the average and range of the mean DPN content of the whole blood and red blood cells were 34.0 (30.0-37.1) and 75.3 (63.4-86.8) μg/ml, respectively. For the last 24 days, the average and range of the mean DPN content of the white blood cells were 87.9 (82.5-94.4) μg/gm and corresponding values for N^1methylnicotinamide of the serum 0.017 (0.003-0.023) μg/ml. Individual subjects maintained fairly uniform levels of DPN concentration for each blood fraction, particularly for the whole blood and the red blood cells. Recoveries of the pure coenzyme from these 2 fractions averaged 100 and 90% respectively. Recoveries of 120% of pure N^1MN and high filtrate blanks in the serum analysis dictated the use of the internal standard method for this fraction. Samples of all fractions except the white cell were stored frozen as the TCA filtrates. Control samples, likewise frozen, were determined daily with each experimental series and provided a valuable means of testing the reliability of the method. The results of this study during a controlled dietary intake suggest that the red blood cell is the most feasible of the blood fractions to investigate for DPN content.

1686. RAT GROWTH EXPERIMENTS WITH 5-HYDROXY-DL-TRYPTOPHAN. Allan J. Morris and Marvin D. Armstrong (intr. by L. T. Samuels). Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.

Growth studies have been carried out with young albino rats in an investigation of the following aspects of tryptophan metabolism: 1) whether dietary 5-hydroxytryptophan (HT) might be of some nutritional significance, in either allowing better growth in the presence of an adequate tryptophan intake, serving as a precursor for tryptophan, or sparing the tryptophan requirement in the manner tyrosine and cystine spare phenylalanine and methionine, respectively; and 2) whether HT is toxic as manifested by poorer growth of animals receiving it. Weanling male albino rats (Sprague-Dawley) were given synthetic diets in which the tryptophan and HT contents were varied. When a diet containing a minimum optimal amount of tryptophan (Oesterling and Rose. J. Biol. Chem. 196:33, 1952) was supplemented with HT there was neither improved nor impaired growth of food consumption until the diet contained 1.0% or more HT; at these levels significantly poorer growth was observed. When portions of the minimum tryptophan requirement were replaced by HT poorer growth resulted,

indicating that HT neither exerts a sparing action for tryptophan, nor is it converted to tryptophan.

1687. DIETARY FACTORS CONTROLLING THE EXCRETORY PATTERNS OF CESIUM-134, POTASSIUM-42 AND RUBIDIUM-86. F. R. Mraz* and H. Patrick. Univ. of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge.

In previous studies, natural feedstuffs were observed to influence the ratios of urinary to fecal cesium and potassium in rats, swine and guinea pigs. This study was inititated to ascertain the factors responsibile for these changes in the excretory patterns of s.c. administered cesium-134, potassium-42 and rubidium-86. A series of experiments were conducted to investigate the influence of alfalfa, Amberlite IRC-50, beet pulp, bentonite and vermiculute at a level of 10% in a simplified (casein-corn starch) diet on the excretory patterns of these radionuclides in the Rockland strain albino rat. The ability of the above materials to remove the radionuclides from distilled water and 0.1 N solutions of potassium chloride, hydrochloric acid, or sodium chloride was also investigated. The experiments demonstrated that the absence of dietary potassium in the basal or vermiculite diet increased urinary and fecal excretion of cesium-134, potassium-42 and rubidium-86, as well as their excretory ratio. Alfalfa, Amberlite IRC-50, beet pulp, bentonite and vermiculite had the property of influencing the excretory patterns of the radionuclides. The magnitude of influence these materials exerted upon urinary and fecal excretion of these radionuclides varied between nuclides. The in vitro data demonstrated that soem of these materials exhibited an affinity for specific nuclides even though these nuclides were in minute quantities in 0.1 N solutions of potassium chloride, hydrochloric acid, or sodium chloride.

1688. RESPONSE OF VITAMIN B_6-DEFICIENT RATS TO GONADOTROPHIC HORMONES. Marjorie M. Nelson, Eloise Wooten* and Herbert M. Evans.* Inst. of Exptl. Biology, Univ. of California, Berkeley.

Previous studies (Wooten et al. 1955) have shown that vitamin B_6-deficient rats required 16 RU of hypophseal follicle-stimulating hormone (FSH) for stimulation of ovarian follicular growth. This amount is 8 times that required by adult hypophysectomized rats of the same body weight. To determine whether similar high levels of other gonadotrophins would also be necessary for normal ovarian development in vitamin B_6-deficiency, the response of such deficient animals to varying levels of hypophyseal interstitial-cell-stimulating hormone (ICSH), human chlorionic gonadotrophin (HCG) and equine gonadotrophins (PMS) has been determined. Stock female rats were placed on the B_6-deficient diet containing the antimetabolite, deoxypyridoxine, at 80-85 days of age and tested after 2 months of deficiency. ICSH and HCG were given s.c. once daily for 4 days; PMS was given in 1 s.c. injection on the 1st day. All rats were autopsied 96 hr after the 1st injection. Injection of 1 or 2 rat units of all 3 gonadotrophins resulted in stimulation of follicular growth and/or in repair of atrophic interstitial tissue in vitamin B_6-deficient animals. These low dosages are similar to those effective in intact immature rats and in hypophysectomized immature or adult rats and are in striking contrast to the high doses of FSH necessary for vitamin B_6-deficient rats. Possible explanations for the difference in response to exogenous FSH and that observed for the other injected gonadotrophins will be discussed. (Aided by PHS Grant A-470.)

1689. DERANGEMENT OF CARBOHYDRATE METABOLISM IN RIBOFLAVIN-DEFICIENT RAT. Mary Ruth Nelson,* Lotte Arnrich* and Agnes Fay Morgan. Dept. of Home Economics, Univ. of California, Berkeley.

Fasting unstressed riboflavin-deficient young rats exhibited gradually falling blood sugar levels after 4 wk on the diet although pair-fed and full-fed control animals had constant or rising levels up to 8 wk and declining values thereafter. The blood lactic acid levels of the deficient rats rose continuously up to 12 wk while those of both riboflavin-fed groups decreased with age. Liver glycogen was always lowest in the deficient and highest in the pair-fed group. Similar groups of normal and riboflavin-deficient rats fasted 48 hr, given a meal of glucose and killed 4 hr later had equal amounts of reducing sugar in the gastrointestinal tract. Nevertheless blood glucose level was increased 3 times as much in the deficient as in the normal and liver glycogen increased 4 times as much in the normal as in the deficient group. Hypoglycemic response to insulin was also greater in the deficient rats but after the 1st hr blood lactic acid was not affected in the deficient group although significantly lowered in the normal animals. Epinephrine treatment produced excessive hyperglycemia in the deficient rats but a similar absolute rise in blood lactic acid levels in both groups. The rate of removal of lactic acid was however slower in the deficient animals and the level at all times remained higher than in the normal rats. It appears that in riboflavin deficiency carbohydrate absorption is normal but its utilization is impaired, with blood and tissue changes in the levels of glucose and its metabolites, glycogen and lactic acid.

1690. SYMPTOMS OF ZINC DEFICIENCY IN THE CHICK. B. L. O'Dell and J. E. Savage.* Dept. of Agricultural Chemistry and Poultry Husbandry, Univ. of Missouri, Columbia.

Five replicates of 10 White Rock males were fed a basal diet containging 25% Drackett protein and a mineral mixture similar to that of Dannenburg et al. (Poultry Sci. 34: 1023) except $ZnCl_2$ was omitted and 0.5% KCl was added. Analysis of the diet indicates that it contains about 50 rpm of zinc. The average weights at 4 wk were: basal, 349; basal plus 6.6 ppm of Zn, 386; basal plus 56 ppm of Zn, 422; and basal plus 5% distiller's dried solubles, 457 gm. Chicks in all groups had low hematorcrits. The ash of distiller's solubles gave no growth response when added to the high level of zinc. The long bones of chicks on the basal diet were shorter and thicker than those on the high zinc supplement. The average ratios of length to diameter of humeri were 8.7 and 9.7; of tibia, 11.4 and 12.4; of metatarsi, 7.3 and 8.5 for basal and supplemented diets, respectively. These differences were statistically significant at less than 1% level. The ash content of dry, fat-free tibia were 45.3% and 46.2%, respectively. Chicks fed a casein or alpha protein type diet (O'Dell et al. Poultry Sci. 31:559), which by analysis contain no more zinc than the Drackett protein diet, showed no response to zinc. The basal itself contained considerable zinc and 6 ppm is the amount commonly supplied in salts mixtures, but it appears that in this diet, zinc is not available to the chick.

1691. COMPOSITION OF DIETARY FAT IN RELATION TO SERUM AND LIVER CHOLESTEROL. Ruth Okey, Marian M. Lyman* and Betty McKee Einset.* Dept. of Home Economics, Univ. of

California, Berkeley.

That differences in the composition of dietary fats may be important in relation to cholesterol absorption and synthesis has seemed likely. The effects of feeding some natural fats of widely differing fatty acid make-up are therefore being investigated. Rats have been given, from weaning, adequate synthetic diets containing 10% of each fat under study. Groups have consisted of 10 males and 10 females each. One group was fed the fat without and another with, added cholesterol. After 7 wk on diet, the rats were autopsied and serum and liver lipids and cholesterols determined. In some cases, cholesterol esters were separated chromatographically and the chain length and degree of unsaturation of the fatty acid moiety studied. With added cholesterol, feeding the fats low in dienoic fatty acids (coconut oil and butter) resulted in lower liver cholesterol values than feeding the fats rich in linoleate (corn, soy and cottonseed oil). Serum cholesterol values and serum phospholipids of the coconut oil-fed rats tended to be high, especially in females. Olive oil which is high in oleic acid, gave comparatively low liver cholesterols, but peanut oil gave fairly high values. There seems to be a tendency toward uniformity of iodine numbers of the fatty acids which were esterified with cholesterol regardless of the iodine numbers of the dietary fat. (Partially supported by Grant H-2152 from Natl. Insts. of Health, PHS.)

1692. EFFECT OF DIETARY FAT, PROTEIN AND CHOLINE UPON SERUM LIPIDS AND LIPOPROTEINS OF THE RAT. Robert E. Olson, John R. Jablonski* and Edmund Taylor.* Dept. of Biochemistry and Nutrition, Grad. School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

Young male albino rats weighing 120 gm were given diets varying in their content of methionine (0.16-0.54%), choline (0-0.3%) and fat (8-42%). The sources of dietary protein were casein and alpha-protein (Drackett) and the sources of dietary fat were lard, corn oil and butter. The total organic sulphur content of each diet was kept constant at 0.2% by supplementation with cystine. The animals were killed after 2 wk of feeding and total liver fat, total serum lipid, total serum cholesterol and total serum lipid phosphorus determined. Both high and low density serum lipoporteins were determined by the technique of Gofman in the analytical ultracentrifuge. It was found that animals fed low-methionine, low-choline diets demonstrated a decrease in all serum lipids, a decrease in the heavy (β) serum lipoproteins and a disappearance of the light (β) serum lipoprotines in association with a fatty liver. This effect was not influenced by the level or kind of dietary fat. The additior of methionine (as casein) to the alipotropic ration prevented the hypolipemia even though it did not, at all levels of dietary fat, prevent fatty infiltration of the liver. Preliminary data in humans fed low protein or alipotropic diets suggest that dietary protein may be a determinant of serum lipid concentrations in man as well as the rat. This finding may have a bearing upon the observation that populations subject to nutritional liver disease have relative freedom from atherosclerosis. (Supported in part by the Vitamin Fndn. and the Dept. of Health, Commonwealth of Pennsylvania.)

1693. SERUM PROTEINS, LIPOPROTEINS AND GLYCOPROTEINS IN MICE WITH HEREDITARY MUSCULAR DYSTROPHY. Hans Oppenheimer,* Alma de Luca* and Ade T. Milhorat. Dept. of Medicine, Cornell Univ. Med. College, New York Hosp., New York City.

The serum proteins, lipoproteins and glycoproteins in mice with hereditary muscular dystrophy (Michelson, Russell and Harman. Proc. Natl. Acad. Sci. 41:1079, 1955) and in their normal litter mates were studied by paper electrophoresis. The protein pattern showed the usual 5 fractions. The relative concentrations of corresponding fractions were alike in both groups of mice. The pattern of the protein-bound lipides consisted of a well-defined band in the albumin region and of a diffuse band spread out among the globulins. The relative concentration of the albumin band for the dystrophic mice (42.7±8.6%) was significantly lower than that for the controls (59.3± 9.3%). The protein-bound carbohydrates were found with the α_1-, α_2- and β globulins (predominantly with α_2 globulin). They seemed to be similarly distributed in the dystrophic and control mice. These results are compared with those of similar studies in nutritional and clinical muscular dystrophy. (Supported by grants from Muscular Dystrophy Assoc. of America, Inc.)

1694. ABSORPTION OF MIXTURES OF ESSENTIAL AMINO ACIDS FROM AN ISOLATED ILEAL LOOP IN MAN. Aline Underhill Orten, Nicholas S. Gimbel* and Arthur H. Smith. Depts. of Physiological Chemistry and Surgery, Wayne State Univ. College of Medicine, Detroit, Mich.

The absorption behavior of varying combinations of the essential amino acids as it occurrs in an isolated intestinal loop in man has been studied. The Moore and Stein chromatographic procedures were used for the analysis of the amino acids. Similar absorption patterns are found both with a protein hydrolysate and with a synthetic L-amino acid mixture containing seventeen L-amino acids in equimolar amounts. The leucine-sioleucine-phenylalanine relationship previously reported persists in large mixtures as well as in a mixture of the 3 alone. The pattern appears to be uninfluenced by the presence of other amino acids. Conversely, the pattern of absorption of tryptophan, lysine, histidine and arginine together or in pairs is not similar to that found in a hydrolystate or the complete amino acid mixture. The addition of either tyrosine or phenylalanine to a mixture of 2 or more of these 4 amino acids either reverses the absorption pattern or brings it to one more closely resembling that found in the complete mixture or in the hydrolysate. (Supported by Grant A-693 from the Natl. Insts. of Health and by a grant from the Detroit Receiving Hosp. Research Corp.)

1695. BIOLOGICAL ESTIMATION OF METABOLIZABLE ENERGY. Bernard L. Oser and Mona Oser.* Food Research Labs., Inc., Long Island City, N. Y.

The difficulties and errors involved in the estimation of the utilizable energy in complex feedstuffs by the presently available techniques of colorimetry, proximate analysis or nutritional balance studies involving extensive mathematical treatment of data, stimulated us to attempt a more direct approach in which the utilizable caloric value of a foodstuff is estimated as a function of the growth of weanling rats under controlled dietary conditions where energy alone is the limiting factor. In practice, weanling rats, are distributed by litters into groups equivalent in sex and weight distribution and are fed a limited quantity of basal diet composed of lactalbumin, vitamins and mineral salts in amounts sufficient to satisfy all the known nutritional requirements of rats except energy. This basal mixture furnishes 9.8 cal/day. To this daily allotment are added 0, 1, 2.5 and 4 gm of sucrose (equivalent to 0, 4-, 10- and 16- calorie

increments, assumed to be completely utilized) or 3.0 gm (dry matter equivalent) of the calorigens under test. In this range the growth response to sucrose is a straight line function and the caloric equivalent of the assay material is interpolated from the curve of response to the sugar. Data will be presented for the development phase of this study and examples of the application of the procedure to the caloric evaluation of various starches and gums will be given.

1696. FACTORS CONTROLLING METABOLISM OF ZINC IN RATS. H. Patrick, J. A. Bacon,* K. N. Carter* and F. Mraz.* Univ. of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge.

Studies on zinc metabolism using radioactive zinc (Zn^{65}) with rats, rabbits and swine demonstrated that vitamin D increased the retention of zinc. High calcium in the presence of normal phosphate uptake also increased the retention of zinc in rats. High magnesium and low phosphorus resulted in increased zinc retention in rats; high phosphorus increased excretion in most studies. Protein or biotin did not appear to influence metabolism of zinc. Bone meal was more active in removing zinc from solution than tricalcium phosphate in vitro.

1697. EFFECTS OF GROWTH PROMOTANTS ON GROWTH AND INTESTINAL WEIGHT OF RATS. Henry S. Perdue,* Henry C. Spruth* and Douglas V. Frost. Abbott Labs., North Chicago, Ill.

One of the mechanisms postulated for the beneficial effect of antibiotics on growth is protection of the intestinal wall to allow better absorption of nutrients. This idea stems from data associating decreased intestinal weight with increased growth. A variety of substances were tested for these effects. Eighteen groups of 10 male weanling rats received a diet of natural feedstuffs with various additions. After 6 wk the rats were weighed and the small intestines of selected groups were excised, washed and weighed. The additions as %, gm intestine/100 gm rat and gm body wt. in order of increasing body wt. were: $CuSO_4 \cdot 5H_2O$, .15%, --, 227; $CuSO_4 \cdot 5H_2O$, .05%, --, 235; Cu-arsanilate, .026%, 2.14,* 236; $CuSO_4 \cdot 5H_2O$, .1%, 1.99,* 237; DL-penicillamine, .008%, 2.12,* 239; Basal, --, 2.56, 240; penicillinase inactivated potassium penicillin G (K-pen-G), .01%, --, 245; penicillin S, .00075%, --, 247; Cu-arsanilate, .013%, --, 253; acid inactivated K-pen-G, .01%, --, 254; K-pen-G, .001%, 2.20,* 259; erythromycin sulfamate, .001%, --, 260; penicillin V, .001%, --, 260; penicillin V, .01%, 2.15,* 265;* erythromycin sulfamate, .01%, 1.88,* 267;* partially purified sodijm synematin B, .1%, 1.99,* 268;* K-pen-G, .01%, 1.97,* 269;* penicillin S, .0075%, 1.80,* 275* (* indicates different from Basal, P=.05). Penicillin V, .01%; erythromycin sulfamate, .01%; synematin B, .1%; K-pen-G, .01%; and penicillin S, .0075%, significantly increased growth and lowered intestinal weight. Copper sulfate, .1%; DL-penicillamine, .008%; and copper arsanilate, .026%, decreased intestinal weight but did not increase growth. This indicates that decreased intestinal weight does not necessarily lead to increased growth. Contrary to findings with other species, copper sulfate, penicillamine and inactivated penicillin did not promote growth of rats.

1698. WEEKLY CHANGES IN HEMOGLOBIN, HEMATOCRIT AND TOTAL PROTEIN IN BLOOD OF CONTROL AND VITAMIN B_6-DEFICIENT PREGNANT AND NONPREGNANT RATS. Ruth L. Pike and Myrtle L. Brown (intr. by Mary L. Dodds). Dept. of Foods and Nutrition, Pennsylvania State Univ., University Park.

One group of rats received 0.8 mg % pyridoxine during pregnancy and another a similar diet with 4 mg % desoxypyridoxine. Two groups of nonpregnant animals received the same diets for an equivalent time. To intensify the deficiency, 2 additional groups received the desoxypyridoxine-containing diet for at least 1 wk before mating. One group then received 0.8 mg % pyridoxine and the other was continued on 4 mg % desocypyridoxine during gestation. Nonpregnant controls were treated similarly. Blood was removed from the tip of the tail on the 1st, 8th, 15th and 21st days. Hemoglobin, hematocrit and total protein were determined from specific gravity of whole blood and plasma. During pregnancy hemoglobin and hematocrit values fail, the greatest decrease occurring in animals receiving desoxypyridoxine either before or during gestation. Plasma protein concentrations increase during the first 2 wk of pregnancy in pyridoxine-deficient animals, followed by a sharp fall in concentration during the last week. Vitamin B_6 deficiency in nonpregnant animals produces an initial rise in hemoglobin and hematocrit, followed by a drop as the deficiency is intensified. Addition of pyridoxine to diets of deficient animals restores hemoglobin and hematocrit calues. Plasma protein concentrations increase as a result of pyridoxine deficiency. The effects of a severe vitamin B_6 deficiency in onpregnant animals are comparable to the effects of a less severe deficiency in pregnant animals. The significance of these results will be discussed. (Supported in part by the Nutrition Fndn.)

1699. DETECTION OF CHANGES IN NUTRITIVE VALUE OF FRYING FATS. C. E. Poling,* W. D. Warner* and E. E. Rice. Research Lab., Swift and Co., Chicago, Ill.

Long periodsof feeding have been used by most investigators studying the changes in fats caused by heating or by heating with aeration in the laboratory. Shorter periods of feeding (7 days) under rigidly controlled conditions have been found to reflect changes in the nutritive value corresponding to those demonstrated by the more elaborate procedures. Using these short term methods the effects of heating several types of oils have been investigated for different laboratory heat treatments. Changes in nutritive value varied greatly and were found to depend to a large degree upon the exact conditions imposed upon the fat. These findings do not justify the extensions sometimes made of laboratory findings regarding the effects of heat treatment of fats to opinions concerning commercial frying operations. However, such data may indicate possibilities which should be investigated.

1700. DISAPPEARANCE OF VITAMIN A IN THE RAT. C. J. Pollard* and J. G. Bieri. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

When vitamin A in aqueous Tween dispersions is injected i.v. into rats, most of the vitamin quickly disappears from the body (Kon et al. Brit. J. Nutrition 9:244, 1955). Present studies indicate that the vitamin is destroyed since none is excreted. The destruction occurs rapidly within the first 2 hr; thereafter very little loss occurs. When 115 μg of vitamin A were injected i.v. into depleted rats weighing 100-120 gm and the animals analyzed periodically for the vitamin, the amounts in the whole animal were: 0 hr., none; 2 hr., 44 μg; 8 hr., 35 μg; 24 hr., 34 μg (single rats). The average amounts in the liver at the same periods were 0 μg, 10 μg, 35 μg and 37 μg, respectively (3 rats per group). Lungs and kidneys

contained from 1-3 μg. In studying the factors involved in the destruction of vitamin A, it has been found that blood of normal rats possesses a system which actively destroys vitamin A. When 1.5-2.0 ml of hemolyzed whole blood were incubated for 2 hr at 37°C with 100-160 μg vitamin A solubilized in saline with Tween 40, from 40-60% of the vitamin was destroyed. The activity is associated with the erythrocytes and is inhibited by -tocopherol, methylene blue, or 0.4 M cyanide. The system appears to decrease with increasing age and is absent in rats over 6 months old. No activity was found in the blood of chicks.

1701. TYPE OF DIETARY FAT AND ESTERIFICATION AND DISAPPEARANCE OF CHOLESTEROL-4-C^{14} IN THE CEBUS MONKEY. Oscar W. Portman and Leonardo Sinisterra (intr. by D. M. Hegsted). Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

The type of dietary fat has previously been shown to influence serum lipid levels in cebus monkeys. Monkeys were maintained on diets supplying 45% of calories as 1) lard (plus cholesterol), 2) corn oil (plus cholesterol) and 3) corn oil (without cholesterol). After 20 wk, when mean values of serum cholesterol were 601, 268 and 237 mg %, respectively, each monkey received i.v. an aqueous suspension of cholesterol-4-C^{14}. After the 3rd day specific activities of the serum total cholesterol declined as a straight line logarithmic function. The mean half-disappearance times for the groups of monkeys were 7.3, 8.4 and 8.8 days. The specific activity of serum ester cholesterol was equal to that of free cholesterol 3 days after injection. Cholesterol esterification was more rapid in the monkeys fed corn oil. De-esterification of cholesterol-4-C^{14}-stearate injected as a suspension was also studied. Analyses of various organs of monkeys indicated that the concentrations of total lipids, lipid phosphorus, or free and total cholesterol were not influenced significantly by the type of dietary fat. Other monkeys were fasted for 24 hr before and after administration by gastric intubation of a small formula meal containing cholesterol-5-C^{14} plus 1 gm of a selected fat or fatty acid. The log disappearance of cholesterol-4-C^{14} from sera consisted of an initial rapid phase (half time-2-9 days) followed by a slow phase (half time-22-35 days). The type of fat used as a vehicle for administration appeared to influence the rate of disappearance of radiocholesterol from the serum.

1702. ESSENTIAL AMINO ACID AND PROTEIN REQUIREMENTS OF THE GROWING RAT. P. B. RamaRao,* V. Chalam Metta* and B. Connor Johnson. Div. of Animal Nutrition, Univ. of Illinois, Urbana.

The high correlation between the biological values of proteins and the indexes calculated from their amino acid contents when whole egg protein is used as standard was reported by Mitchell and Block (J. Biol. Chem. 163:599, 1946). The actual requirements for the essential amino acids and total protein might be expected to be a better primary standard for such protein evaluation. The requirement for each of 9 essential amino acids for growth and nitrogen retention on a diet containing 5% casein (for palatability) was determined. Each essential amino acid (L-form) was tested at various levels using as reference point the values of Rose et al. (J. Biol. Chem. 181:307, 1949) or of 10% whole egg protein, whichever was lower. Nonessential amino acids were added to adjust the total protein to the desired level. The requirements found for growth, expressed as percentage of the diet are lysine 0.9, tyrptophane 0.15, isoleucine 0.55, valine 0.55, methionine (+ cystine) 0.5, threonine 0.5, leucine 0.7, phenylalanine (+ tyrosine) 0.7 and histidine 0.25. With these levels optimum growth (4.5 gm/day) was obtained at 8% protein (N x 6.25) in the diet, and optimum nitrogen retention was obtained at 10%. Growth and nitrogen retention were not improved above these levels by increasing the total protein of the diet either by raising the level of the nonessential amino acid mixture used or by increasing the levels of both the essential and the nonessential amino acid mixtures.

1703. EFFECTS OF INCORPORATING LOW LEVELS OF SODIUM FLUORIDE IN DRINKING WATER OF ALBINO RATS. W. F. Ramseyer (intr. by C. M. McCay). Research Labs., Swift and Co., Chicago, Ill. and Dept. of Animal Husbandry, Cornell Univ., Ithaca, N. Y.

Albino rats that had received levels of 0, 1, 5 and 10 ppm of fluorine as sodium fluoride in the drinking water from the time of conception of the parent until killed at 150 and 520 days of age were examined for possible physical, pathological and chemical changes in the various tissues. Dental observations did not reveal any changes in the molars of those killed at 150 days of age, however, in the older animals an increase in the number of carious teeth and periodontol lesions was observed with an increase in the amount of fluorine ingested. A small number of 520-day old animals was randomly selected for kidney examinations and an apparent renal tubule hypertrophy and tubule regeneration was noted in those animals receiving fluorine. An estimation of the amount of fluorine in the femur indicated an increase in the amount of fluorine in the bone with an increase in the amount of fluorine ingested although there are apparently large individual differences relative to the storage of fluorine in the bone. With the 2 higher levels of fluorine, the femur of the older animals contained a larger amount of fluorine than did the femur of the younger animals. Apparently this level of fluorine as sodium fluoride in the drinking water had no significant effect on the body weight; the weight of the femur or the density of the femur of albino rats.

1704. COMPARATIVE GROWTH RESPONSE OF RATS ON NATURAL AND RACEMIC AMINO ACIDS. M. Rechcigl, Jr.,* J. K. Loosli and H. H. Williams. Depts. of Biochemistry and Nutrition and Animal Husbandry, Cornell Univ., Ithaca, N. Y.

This investigation was made in order to obtain information regarding the nature of the growth inhibition of rats fed racemic amino acids. The basal nitrogen-free diet (1) was compared with diets (2-7) supplemented with mixtures of essential amino acids including cystine and tyrosine, patterned after the composition of rat carcass (J. Biol. Chem. 208:277, 1954). All amino acids were present in the L form in diet 2 and in the DL form in diet 3, except for L-arginine and L-cystine. Diet 4 contained amino acids as in diet 2 except that the level of each was approximately doubled. Diet 5 contained an isonitrogenous level of DL-amino acids comparable to diet 4. Diets 6 and 7 were the same as diet 5 except that tyrosine in diet 6 and isoleucine, leucine and valine in diet 7 were present in the L form. At the end of 10 days the average gains of rats (in gm) for the respective diets were as follows. In the 1st experiment: diet 1, -13; 2, 12; 3, -12; 4, 22.5; 5; -7. In the 2nd experiment: diet 5, -2; 6, -2.5. In the 3rd experiment: diet 1, -12; 7, 19. In view of the recently reported antagonism between leucine and isoleucine (Arch. Biochem.

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Biophys., 57:1, 1955) it is suggested that the growth inhibition was due to a deficiency of isoleucine which is aggravated by an excess of leucine in the diet. (Supported by a grant from the Herman Frasch Fndn.)

1705. EFFECT OF COMMERCIAL FRYING OPERATIONS ON NUTRITIVE VALUE OF FATS. E. E. Rice, P. E. Mone* and C. E. Poling.* Research Lab., Swift and Co., Chicago, Ill.

Thirty-four samples of fats obtained from restaurants, bakeries and producers of potato chips and doughnuts have been studied to determine the influence of the various usages on nutritive value. Even the most severely used of these commercial fats caused only slight decreases in the calorie availability of the fats and slight increases in liver size. These findings do not support the speculation that the fats or prepared foods may be seriously impaired in value or that they may actually be harmful, an opinion frequently expressed as a result of studies of highly unsaturated fats heated under laboratory conditions. Instead they suggest that commercial fats are more resistant to change nutritionally and that conditions of commercial usage, e.g. heating, aeration, oil turnover, etc. are in effect much less drastic than laboratory conditions often selected arbitrarily.

1706. COMPARISON OF ION EXCHANGE AND MICROBIOLOGICAL PROCEDURES FOR DETERMINATION OF FREE AMINO ACIDS IN BLOOD PLASMA. L. R. Richardson and Patricia W. Lauderdale.* Texas Agricultural Exp. Station, College Station.

Blood taken from pigs receiving 3 diets was heparinized and centrifuged to separate the plasma. The animals were fasted 18 hr before blood was collected. A picric acid protein free filtrate was prepared from the plasma for 4 determinations each by ion exchange and microbiological procedures. Seventeen free amino acids were determined in the protein-free filtrate by the ion exchange method of Moore and Stein (J. Biol. Chem. 211:893, 1954). Eleven of the same amino acids were determined by microbiological procedures. The values by the 2 procedures agreed for all amino acids, except lysine, histidine and tryptophan. An example of the average values, in mcg/ml of plasma, by ion exchange and microbiological procedures, respectively, were lysine 113.9 and 21.5; histidine 40.8 and 27.8, tryptophan 38.6 and 6.3. A fraction containing the lysine peak from the ion exchange separation of this same filtrate assayed by the microbiological procedure, gave a value of 23.5 mcg/ml of plasma. These data and the shape of the lysine peak indicate that the values obtained by the ion exchange method for lysine may not be reliable. Similar comparisons will be reported for histidine and tryptophan. Duplicate values were obtained for 17 free amino acids in blood plasma of 6 pigs receiving the same diet. Variation between duplicate values for plasma from the same animal were small, but the variations for different animals were relatively large.

1707. INFLUENCE OF DIETARY PROTEINS DURING PREGNANCY ON RAT LIVER COMPOSITION. Charlotte Roderuck and Janice Godwin.* Home Economics Research, Iowa Agricultural Exp. Station, Ames.

Weanling female rats were fed diets consisting of dried pork equivalent to 15% pork proteins, 5% torula yeast, 22% fat, 4% Osborne and Mendel salts, 2% ruffex, 1% NaCl and cornstarch to make to 100%. At 8 wk of age, they were mated and continued on the same diet or on one with the following substitutions for the 15% pork proteins: 10% pork proteins, 15% pork proteins plus vitamin B_{12}, 15% pork proteins plus methionine and phenylalanine, 15% lactalbumin proteins, 15% lactalbumin proteins plus vitamin B_{12}, or 15% lactalbumin proteins plus several amino acids. Rats fed the stock colony ration throughout grwoth and pregnancy served as controls. First litter performance was recorded and the animals mated again. On the 21st day of the 2nd pregnancy, livers and fetuses were removed and weighed. Livers were analyzed for total fat, nitrogen, moisture and choline. Total liver fat amounted to nearly 30% of the wet liver weight in pregnant rats fed diets containing lactalbumin proteins, to 10-20% in rats fed pork proteins and to 4% in the control rats. Amino acid additions, but not vitamin B_{12}, reduced the liver fat of the experimental animals to that of the controls. Total liver nitrogen and choline remained relatively constant throughout the experimental groups. The number and size of the embryos were not related clearly to changes in liver lipids.

1708. ETHIONINE AS AN ANTIMETABOLITE FOR METHIONINE AND CHOLINE. W. D. Salmon and D. H. Copeland.* Dept. of Animal Husbandry and Nutrition, Agricultural Exp. Station, Alabama Polytechnic Inst., Auburn.

In 1938 Dyer reported that ethionine acted as an antimetabolite for methionine (J. Biol. Chem. 124:519). Several investigators have since used ethionine for this purpose. Levine and Tarver reported that when ethionine labeled in the ethyl group was fed to rats the labeled analogue was taken up in tissue proteins, thus forming abnormal proteins (J. Biol. Chem. 192: 835, 1951). Popper et al. reported hyperplastic nodules, bile duct proliferation and cholangiofibrosis in livers of rats fed 0.2-0.5% of ethionine for 51-208 days. Farber has reported liver carcinoma in rats fed ethionine for 8-11 months. Stekol and Weiss have shown by use of labeled ethionine and existence in rats of a pathway for deethylation of ethionine and have suggested that triethylcholine and other ethyl analogues may account for the growth inhibition effect of ethionine (J. Biol. Chem. 85:577, 1950). The addition of 0.05-0.2% of ethionine to diets containing 0.2% or less of choline chloride for 7-wk old rats markedly inhibited growth. The effects of ethionine were appreciably antagonized, however, by supplemental methionine or choline. The antagonistic action of methionine or choline against ethionine was synergized by vitamin B_{12}. Ethionine was amuch more effective antimetabolite for methionine or choline than was triethylcholine. The lesions produced by ethionine feeding resemble those of choline deficiency, including liver cirrhosis, cholangiofibrosis and hepatocellular carcinoma. (Supported in part by grants from the American Cancer Society and the Natl. Cancer Inst., PHS.)

1709. IMMUNOCHEMICAL AND ENZYME STUDIES ON MYOSIN FROM DENERVATED AND NORMAL MUSCLE. Arthur Samuels (intr. by F. A. Cajori). Chemical Embryology, Univ. of Colorado Med. Ctr., Denver.

Two rabbit antisera prepared against 3 times reprecipitated myosin from chicken leg muscle, yielded quantitative precipitin curves that showed single sharp peaks, indicating a single antigen-antibody system. Myosin prepared from denervated gastrocnemii embryonic leg or heart gave precipitin curves with lower slopes and of significantly less height than myosin prepared from the normal contralateral gastrocnemii or the antigen. Preliminary tests showed that these antisera could be absorbed with the test myosins

and still give a precipitin reaction with normal adult myosin. These results indicate that the structure of denervated and embryonic myosin may differ from normal myosin. A similar conclusion has been drawn on the basis of birefingence of flow studies (Dreyfus et al. Compt. Rend. Acad. Sci. 236:2351, 1953). However, later attempts to repeat this work with other rabbits and myosin preparations which seemed to appear as a single component during prolonged electrophoresis at 3 pH values, yielded several antisera which show precipitin curves with broad peaks and usually 2 maxima, with which the denervated muscle myosin always reacts to a greater extent than normal muscle myosin. Agar diffusion tests confirmed the presence of at least 2 systems. To discover the identity of the contaminating antigens, enzymatic studies on the myosins were initiated. These show that although myokinase appears to be absent, adenylic deaminase is present in varying amounts. The existence of the latter as a contaminant and not as part of the myosin molecule is indicated by experiments which differentially fractionate the deaminase between the "actomyosin" (actin poor myosin) and the more soluble myosin as the former precipitates in larger amounts and deaminase activity with decreasing ionic strength.

1710. RELATIONSHIP OF HYDROXYLYSINE IN MICROBIOLOGICAL ASSAYS FOR LYSINE. H. E. Sauberlich. Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.

Studies were made on the interference of hydroxylysine in the microgiological assay of proteins for lysine. The organisms employed were Leuconostoc mesenteroides P-60 and a strain of Streptococcus faecalis R. Analyses of a series of proteins for lysine with L. mesenteroides revealed that the presence of hydroxylysine in the sample may cause a considerable error in the values obtained. Hydroxylysine spared the requirement for lysine, but did not replace the lysine of L. mesenteroides. When hydroxylysine was added to the culture medium (0.5 mg/ml), a very marked improvement was obtained in the lysine response curve. Comparison of values obtained by analysis with medium free of hydroxylysine with that of medium containing hydroxylysine revealed marked discrepancies with certain samples. Gelatin and egg albumin appeared to contain appreciable amounts of hydroxylysine, whereas peanut meal and soybean meal were low or devoid of this amino acid. With the procedure employed, S. faecalis failed to grow unless hydroxylysine was present in the culture medium or high levels of lysine were employed. When hydroxylysine was present in the medium, S. faecalis was a satisfactory assay organism for lysine, either as a turbidimetric or titrimetric procedure. Values obtained with S. faecalis corresponded to those obtained with L. mesenteroides when hydroxylysine was present in the assay medium. Both organisms demonstrated an amino acid imbalance relationship between lysine and hydroxylysine. Radioisotope studies on the relationship of lysine to hydroxylysine will be presented.

1711. VALINE AND HISTIDINE REQUIREMENT OF THE NORMAL INFANT. Selma E. Snyderman. L. Emmett Holt, Jr., Patricia M. Norton,* Florence Smellie* and Audrey Boyer.* Dept. of Pediatrics, New York Univ. College of Medicine, New York City.

The valine requirement has been determined for male infants 1-3 months of age. This was accomplished by the use of a synthetic diet, the protein moiety of which is composed of 18 L-amino acids provided in the same proportions as breast milk. After a control period, the valine was completely withdrawn from this mixture and then reintroduced in stepwise fashion until normal growth was obtained. The quantity of nitrogen in the diet was kept constant by the substitution of glycine. Our criteria of normal growth included weight gain, nitrogen retention and plasma protein values. An intake of approximately 85 mg/kg/day of valine was sufficient to allow good weight gain and nitrogen retention and to maintain the plasma proteins. This is less than 1/2 the amount contained in usual infant formulas. Urinary excretion of free amino acids as determined by paper chromatography revealed a fall in valine excretion during periods of deficiency. Similar studies carried out on 2 infants with histidine indicate that histidine is an essential amino acid for the growing child.

1712. INFLUENCE OF ADDITIONS OF SMALL AMOUNTS OF DEFATTED FISH FLOUR TO PROTEINS IN MILLET AND GRAIN SORGHUM ON GROWTH AND PROTEIN EFFICIENCY. Barnett Sure. Dept. of Agricultural Chemistry, Univ. of Arkansas, Fayetteville.

A study was made on the influence of additions of 1, 3 and 5% defatted fish flour (mixture of carp, smelts and whitings) to the proteins of millet and grain sorghum on growth and protein efficiency ratio (PER) expressed as gains in body weight per gram of protein intake. This investigation was carried on the albino rat during a 6-wk period. The results are expressed percentagely as increases in body weight (B.W.) and on PER. MILLET. 1% fish flour: +31.3% in B.W. and +8.6% in PER; 3% fish flour: +117% in B.W. and +26% in PER; 5% fish flour: +343.2% in B.W. and +98.1% in PER. GRAIN SORGHUM. 1% fish flour: +193.7% in B.W. and +128.3% in PER; 3% fish flour: +409.5% in B.W. and +186.8% in PER; 5% fish flour: +644.4% in B.W. and +213.2% in PER. Since millet and grain sorghum are used extensively in great parts of the world, these results may have applications in feeding malnourished nations ni Asia, Africa and in Latin American countries where there is a marked prevalence of the protein deficiency disease, Kwashiorkor.

1713. CERTAIN ANTICARIOGENIC FACTORS IN OAT HULLS. Fumito Taketa* and Paul H. Phillips. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

The acidic fraction from ethanol extracts of oat hulls has shown promise as an anticariogenic agent in the control of experimental caries in the cotton rat. Further purification of this fraction has yielded saturated fatty acids and a group of phenolic acids possessing inhibitory effects on organisms isolated from the carious molars of cotton rats and on pure strains of lactic acid producing bacteria. The phenolic acids have been separated by 2-dimensional paper chromatography using iso propyl alcohol-ammonia-water (8:1:1) and 20% aqueous KCl as the developing solvents. At least 10 compounds reacting with diazotized sulfanilic acid and with the Folin-Ciocalteau phenol reagent have been resolved. The major components have been tentatively identified as vanillic, homovanillic, ferulic and p-phyroxybenzoic acids. These as well as related compounds are under study in the control of experimental caries in the cotton rat and their effect on microorganisms.

1714. INFLUENCE OF COBALTOUS CHLORIDE ON AORTIC ATHEROMATOSIS AND PLASMA LIPID PATTERN IN CHOLESTEROL-FED CHICKENS. David M. Tennent,* Charles W. Mushett,* Gunther

W. Kuron,* Walther H. Ott and Henry Siegel.* Merck Inst. for Therapeutic Research, Rahway, N. J. and Pathology Dept., Albert Einstein College of Medicine, New York City.

In experimental atherosclerosis produced by feeding cholesterol and cottonseed oil to White Leghorn cockerels the addition of 0.5% $CoCl_2 \cdot 6H_2O$ to the diet resulted in decreased incidence and severity of aortic lesions and improved plasma lipid pattern. Cobaltous chloride in this amount was toxic and its feeding caused considerable weight loss. $CoCl_2 \cdot 6H_2O$ at a concentration of 0.05% in the diet had little effect on growth, but again reduced the incidence and severity of aortic atherosclerosis and improved the plasma lipid pattern. It produced no morphological changes in the pancreas. Parenteral administration of cobaltous chloride in various amounts did not reduce atheromatosis or improve the plasma lipid pattern. These results suggest that dietary cobalt lowers the plasma cholesterol concentration and decreases atheromatosis by interfering with the absorption of cholesterol from the diet.

1715. INTESTINAL ABSORPTION OF FREE FATTY ACIDS. H. C. Tidwell. Dept. of Biochemistry, Univ. of Texas, Southwestern Med. School, Dallas.

To gain further insight into the major form in which fatty acids are absorbed, attempts have been made to follow the rates of absorption of the various glycerides, the effects of their state of hydrolysis or emulsification in the intestinal tract and the absorption of free fatty acids when ingested as such. After ingestion of 0.3 ml of olive oil (TG) per sq dm of body surface or its fatty acid (FA) equivalent of diglyceride (DG) or monoglyceride (MG), the readily hydrolyzable TG and DG gave the highest particulate blood fat levels. The MG and FA gave lower levels as a result of gastric retention. Similar blood fat levels were obtained after the ingestion of TG and FA when the intake was reduced to 0.1 ml/sq dm. The rate of absorption appeared from evidence obtained to be related more to degree of hydrolysis than to the state of emulsification. Some amino acids which markedly increased the rate of fat hydrolysis, also promoted its absorption. The rate of absorption of FA at both levels equaled that of the ingested TG if the lipid considered was only that part which was not retained in the stomach. These results support the contention that a large part if not all glycerides may be hydrolyzed to fatty acids before absorption. The possibility of complete interchanges of glyceride fatty acids in the lumen as well as a reported selective absorption of free fatty acids prevents even labeling techniques from supplying indisputable evidence of the form absorbed. (Supported in part by grants from Robert A. Welch Fndn. and the PHS.)

1716. ENZYMATIC PATHWAY FROM IODINATED THYRONINES TO THEIR CORRESPONDING THYROACETIC ACIDS. Kenkichi Tomita (intr. by Clark Gubler). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

Thyroxine and triiodothyronine are converted to their corresponding acetic acid analogues by an extract of rat kidney mitochondria (Tomita, Lardy, Larson and Albright. J. Biol. Chem. 224, in press, 1957). Possible intermediates in this conversion are the corresponding thyropyruvic acids or, the corresponding thyronamines. No evidence has been obtained for conversion of the latter to thyroacetic acid derivatives. The difficulty of obtaining tri- or tetraiodothyropyruvic acids led us to study the corresponding reactions with the diiodo compounds. Diiodothyropyruvic acid labeled with C^{14} in the carbonyl position was synthesized from methylene-labeled glycine. The radioactive diiodothyropyruvic acid is converted by enzyme preparation to radioactive diiodothyroacetic acid. The pyruvic anc acetic analogues were separated by paper chromatography using tertiary amyl alcohol saturated with 0.1 M phosphate buffer pH 6.4 (R_f 0.38 and 0.81, respectively). Short time incubations resulted in the accumulation of a compound migrating at an intermediate rate. This component disappears from the reaction mixture after 2 hr as the accumulation of diiodothyroacetic acid is greatly enhanced. From these model studies, it seems likely that triiodothyropyruvic and tetraiodothyropyruvic acids are intermediates in the conversion of triiodothyronine and thryoxine to their corresponding acetic acid analogues.

1717. INTERRELATIONSHIP BETWEEN ASCORBIC ACID AND VITAMIN E IN IRON ABSORPTION AND METABOLISM IN THE RAT. Robert G. Tucker,* Arthur E. Heming,* Joyce K. Mathues* and Samuel M. Greenberg. Smith, Kline and French Labs., Philadelphia, Pa.

To determine the effects of ascorbic acid, vitamin E and N, N'-diphenyl-p-phenylene-diamine (DPPD) on iron absorption and metabolism, 4 experiments were performed on 4 different samples of iron-deficient rats receiving a low vitamin E diet. 1) A group of rats was observed for 3 wk. For 5 days of each week all received daily doses of either 2 mg or 4 mg Fe/kg. In addition, 1 subgroup received vitamin E; 1 subgroup, ascorbic acid; and 1 subgroup both. Those animals which received both vitamins showed the greatest increase in hemoglobin regeneration. 2) Three doses of ferrous sulfate (1.5 mg; 3.0 mg; and 6.0 mg Fe/kg) were given for 4 wk. Results similar to the 1st experiment were obtained. 3) Massive doses (25 mg Fe/kg) were given 3 times in 10 days. Hemoglobin regeneration rates were similar for the 4 subgroups. The hemoglobin level attained by iron supplementation was maintained for 7 wk in the rats who also received both bitamins. In the other subgroups the levels declined beginning 2 wk after the last iron dose. These observations indicate a probable interrelationship between the 2 vitamins not only in iron absorption but also in iron metabolism. 4) The same as experiment 3, but using DPPD (an antioxidant) in place of vitamin E. DPPD gave results similar to those observed with vitamin E.

1718. EFFECT OF FEEDING AN ATHEROGENIC DIET ON MAGNESIUM REQUIREMENT. Joseph J. Vitale,* P. L. White,* M. Nakamura* and D. M. Hegsted. Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.

The addition of thyroxine to the diet of rats results in an inhibition of growth and a decrease in the serum magnesium levels, both of which are partially overcome by feeding excess magnesium. These observations together with the known effect of thyroxine on the level of serum cholesterol led us to investigate the effect of feeding an atherogenic diet on Mg metabolism. A 10% protein and 20% fat diet, with and without cholesterol (1%) and cholate (.3%) and containing various levels of Mg (12, 24, 48, 96 and 192 mg %) was fed to weanling rats for 24 days at which time blood samples were drawn and the animals killed for histological study. The control animals fed the diets containing no cholesterol and cholate grew maximally when fed 24 mg % Mg, had an average serum Mg level of 1.9 mg %, had no morphological changes in any of the organs examined and had serum cholesterol concentrations

ranging from 90 to 110 mg %. Animals fed the diets with cholesterol and cholate did not grow maximally, had low levels of serum Mg, high levels of serum cholesterol (639-808 mg %), and had an extensive heart score (Sudanophilia of heart and aorta). Increasing the Mg content of the diet to 192 mg % largely overcame all of these effects except the level of serum cholesterol which remained high. It is concluded that the atherogenic diet used in these studies produces a magnesium deficiency accompanied by hypercholesteremia and an extensive Sudanophilia of the heart and aorta. (Extensive kidney lesions were also produced in these rats, see abstract by E. Hellerstein and N. Zamcheck.)

1719. SOME ASPECTS OF TRYPTOPHAN METABOLISM ON LOW NIACIN INTAKE. Virginia M. Vivian,* Marilyn M. Chaloupka* and May S. Reynolds. Dept. of Foods and Nutrition, School of Home Economics, Univ. of Wisconsin, Madison.

Following a 6-day control period on ordinary foods providing daily about 650 mg tryptophan, 10 mg of niacin, 10 gm nitrogen and generous amounts of all other nutrients, 4 women were maintained for 30 days on a semi-synthetic diet in which foods provided approximately 2.5 mg of niacin and 30 mg of tryptophan. After the initial 6-day period on the 30-mg tryptophan intake, supplements of l-tryptophan given during successive 6-day periods brought the total intakes to 150, 190, 270 and 670 mg/day, respectively. Niacin intake remained constant throughout the experiment. Blood pyridine nucleotides (PN), urinary N-Methyl 2 pyridone-5-carboxamide (6-pyridone) and nitrogen balance were determined. On the first 2 periods of the semi-synthetic diet, mean blood PN levels decreased 20-40% below control period levels; showed a definite increase on 270 mg and returned to the level of the control period on an intake of 670 mg. The 6-pyridone decreased rapidly on the 30-mg intake and continued decreasing until little if any was detected on the 270-mg intake. The first definite increase occurred on the 670-mg intake. These data suggest that under conditions of this experiment, tryptophan was used preferentially to establish nitrogen balance before any synthesis of niacin became evident. This synthesis appeared to take place at a tryptophan intake between 190 and 270 mg.

1720. EXCRETION OF FIVE ESSENTIAL AMINO ACIDS BY YOUNG WOMEN. F. Waddill,* R. M. Leverton and M. Skellenger.* Nebraska Agricultural Exp. Station, Lincoln.

Urinary excretion of L-threonine, L-valine, L-tryptophan, L-phenylalanine and L-leucine was determined microbiologically for the young women who were subjects in the studies of the quantitative requirements for these amino acids (J. Nutrition 58: 59, 83, 219, 341 and 355, 1956). Each subject was studied on several levels of intake and individual variations in excretion were high. There was a tendency for the urinary excretion of an amino acid to increase as its intake was increased. These increases in excretion were small when compared with the size of increases in intake. In general, on intakes of an amino acid which caused negative nitrogen balance there was an increase in the percentage of the amino acid which was excreted. No relationship between body weight or size and the amino acid excretion was found. (Supported in part by a U.S. Dept. of Agriculture contract, sponsored by the Human Nutrition Branch, Agricultural Research Service.)

1721. ROLE OF VITAMIN B_{12} IN NUCLEIC ACID SYNTHESIS. S. R. Wagle* and P. Connor Johnson. Lab. of Animal Biochemistry, Univ. of Illinois, Urbana.

It is well established that formate, glycine, serine and the methyl group of methionine serve as precursors in the biosynthesis of purines and pyrimidines which are then incorporated into RNA and DNA. Vitamin B_{12} has been postulated to be involved in this synthesis. Formate-C^{14}, formaldehyde-C^{14}, glycine-2-C^{14}, serine-3-C^{14} and methionine methyl-C^{14} were injected into vitamin B_{12}-deficient and B_{12}-normal chicks and baby pigs. Similarly, glucose-C^{14} uniformly labeled was injected into deficient and normal baby pigs. Animals were killed after 4 hr. Both RNA and DNA were isolated from the livers and the radioactivity was determined. The total radioactivity of the RNA and of the DNA isolated from the formate- and formaldehyde-injected animals was higher than that of the RNA and DNA isolated from the animals which received the other precursors (glycine, serine and methionine). However, in no case was any difference found between B_{12}-deficient and the B_{12}-normal animals. Similarly, no difference in activity was found in RNA and DNA isolated from the livers of the pigs (both deficient and normal) injected with radioactive glucose. These results suggest that vitamin B_{12} is neither involved directly in the incorporation of 1-carbon precursors into nucleic acids, in the conversion of glucose to ribose, nor in the incorporation of ribose into nucleic acids. The results obtained were similar for both pigs and chicks.

1722. AVAILABILITY OF ENERGY AND ESSENTIAL AMINO ACIDS IN EGG, PORK AND PEANUT BUTTER. Johnnie H. Watts, Lovie K. Booker, Janet V. Wilson, E. Geraldine Williams and Walter G. Wright (intr. by Helen G. Oldham). Carver Fndn., Tuskegee Inst., Ala.

Six adult male students were experimental subjects during 4 dietary periods. The intakes during these periods consisted of a very low nitrogen diet (control), the low N diet + whole egg, and low N diet + pork muscle and the low N diet + peanut butter. Ninety-four percent of the N intake during the latter 3 periods came from 1 of the protein test foods. The 4 diets contained approximately the same number of calories. In the 3 diets which contained a protein food, protein was included at the level of 1 gm/kg/day. Nitrogen balances were determined. Caloric determinations and microbiological assays for essential amino acids were made on intakes and fecal collections. The availability of food energy and the apparent digestibility of essential amino acids were calculated for the total diets and for the test foods. The amounts of available energy in the egg, pork and peanut butter diets were 94.8, 95.2 and 93.6%, respectively. Values for the apparent digestibility of essential amino acids in the egg and pork diets were quite similar, ranging in the egg diet from 88.2% for tryptophan to 95.5% for methionine and in the pork diet from 86.6% for tryptophan to 93.5% for leucine. Values for the apparent digestibility of the essential amino acids in the peanut butter diet were lower, ranging from 74.3% for methionine to 91.2% for phenylalanine.

1723. ENERGY REQUIREMENTS OF MEN IN SUB-ARCTIC BIVOUAC. B. E. Welch, L. M. Levy, C. Frank Consolazio, E. R. Buskirk and T. Dee. (intr. by Theodore E. Friedemann). U.S. Army Med. Nutrition Lab., Fitzsimons Army Hosp., Denver,

Colo. and Quartermaster Research and Development Command, Natick, Mass.

A group of 26 healthy soldiers were placed on the Army C-ration for a period of 37 days. The first 8 days were a control period (I) at Natick, Massachusetts; the second 8 days a prebivouac period (II) at Fort Churchill, Canada; and the remaining 21 days divided into 3 bivouac periods (III, IV, V) of 7 days each. During the bivouac period the men were entirely self-sufficient with respect to transportation, clothing, shelter, food, etc. from site to site. The men covered some 8-12 miles/day and established a new bivouac site each night. Body weight measurements were taken daily (prebreakfast, nude). Measurements followed on a period basis included food intake, water intake, urine output, fecal output, nitrogen balance, total body water, body density and modified Harvard Step Test (3 min test) score. The caloric intake at Natick (I) was 2536 cal., the lowest of the periods studied. As the activity was increased, the caloric intake increased, reaching a high of 4488 cal. during the 3rd bivouac (V). The bivouac periods (III, IV, V) averaged 4196 cal/day. Average total body water and body weight showed a decrease during the course of the test. Body density increased during the bivouac periods. The influence of the change in these measurements upon the caloric requirement in this experimental regimen will be discussed.

1724. INFLUENCE OF COPROSTANOL FORMATION ON CHOLESTEROL ABSORPTION. W. W. Wells (intr. by A. E. Axelrod). Biochemistry Dept., School of Medicine, Univ. of Pittsburgh, Pittsburgh, Pa.

Dietary variables have previously been demonstrated to influence the extent of coprostanol formation from dietary cholesterol in a number of species. Studies have been in progress to correlate the elevation of liver and blood cholesterol of both rats and rabbits on 0.25% and 0.5% cholesterol diets with the failure of intestinal anaerobes to hydrogenate cholesterol to coprostanol. Groups of both young and adult rats were fed fat-free diets containing sucrose or lactose at different levels and supplemented with 0.25% cholesterol. Blood and liver samples were analyzed for free and total cholesterol and pooled fecal pellets removed from the colon were assayed for cholesterol and coprostanol correcting for accompanying neutral sterols by chromatographic and colorimetric techniques. The blood and liver cholesterol levels of young rats fed high lactose diets were not significantly higher than the sucrose fed controls. Coprostanol formation was found to be low in the young controls while in the lactose fed rats, the coprostanol formation was only somewhat lower. In contrast, adult rats on 30% lactose for 2-3 wk or 60% lactose for 6 days responded with marked inhibition of coprostanol formation accompanied by elevated liver cholesterol. Rabbits fed 0.5% cholesterol, 20% lactose diets developed a pattern of blood and liver cholesterol and fecal cholesterol and coprostanol analogous to that found in adult rats. The effects of dietary lipids in contrast to lipid free diets will be discussed.

1725. TOXICITY OF ORGANIC ARSENICALS WHEN FED TO CHICKS. F. D. Wharton, Jr.,* R. E. Schoene,* M. J. Smidt* and J. C. Fritz. Dawe's Labs., Inc., Chicago, Ill.

Arsonic acid derivatives have been used widely in manufactured feeds for growth stimulation and for control of disease. For 4-amino phenylarsonic acid there are available in the literature a number of comprehensive reports regarding tolerance levels. These studies were undertaken in an effort to develop data on the tolerance for other organic arsenicals when used continuously in the feed. In a chick growth test, 4-hydroxy phenylarsonic acid was added to a practical starting ration at levels of 0, 35.5, 71.0, 142.0, 177.6 and 355.1 gm/ton. No harmful effects were observed and even the highest level appeared to stimulate growth. A series of tests were made with 3-nitro-4-hydroxy phenylarsonic acid at levels of 0, 45, 90, 135, 180, 225, 270 and 450 gm/ton of complete ration. Above about 90 gm/ton, this arsonic compound depressed growth. When the quantity in the ration was 180 gm or more per ton, some nervous symptoms were observed. These included an unusual walk, reluctance to stand and nervousness when excited. Only at the highest level fed was there any mortality which could be blamed upon the arsonic compound. The possibility that other dietary components might influence the toxicity of the arsenical was investigated. Inconclusive results were obtained with p-amino benzoic acid and with methionine. Restricting water access to 1 hr each in the morning and in the afternoon, with feed before the chicks at all times, did not accentuate toxicity symptoms.

1726. RELATIONSHIPS OF PROTEIN AND FAT IN PYRIDOXINE DEFICIENCY. Mary Ann Williams and Nina L. Cohen (intr. by Ruth Okey). Dept. of Home Economics, Univ. of California, Berkeley.

Feeding fat reportedly ameliorates the development of pyridoxine deficiency. This may reflect a) a function of the fat itself, or b) an indirect effect resulting from the fact that, if the ratio of protein/food energy in the test diets is not constant, the animal eating the high fat diet will eat less protein and therefore need less pyridoxine for its metabolism. To test the latter hypothesis, diets in which the ratio/protein/food energy was held constant but the fat content was varied from 0 to 40% were fed to male weanling rats for 3 wk. In a control experiment, the ratio of protein/food energy was not adjusted when the level of fat was increased from 0 to 40%. Vitamin supplements were fed separately. The fat-soluble vitamins were supplied in cottonseed oil, which furnished approximately 10 mg of linoleic acid per day. Pyridoxine-supplemented animals were pair-fed with the deficient groups. Cottonseed oil and coconut oil were the fat sources. With either oil, levels of dietary fat above 5% had no significant effect on the development of pyridoxine deficiency when the protein/food energy ratio was constant. However, if this ratio was decreased, the 40% cottonseed oil diet permitted slightly better growth, but the difference was not significant. The composition of the body tissue of the pyridoxine-deficient and control animals will be reported. (Supported by Grant A-1189, M&N, Natl. Insts. of Health, PHS.)

1727. NUTRITIONAL STUDIES WITH ETHANOLAMINE IN THE CHICK. Robert L. Wixom,* George E. Pipkin,* John H. Wikman* and Paul L. Day. Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.

In view of recent radioactive tracer evidence for the conversion of ethanolamine to glycine in the rat (Weissbach and Sprinson. J. Biol. Chem. 203:1023, 1031, 1953), the following growth experiments were performed with 1-wk-old chicks. Each test substance was added at a level equimolar to 1.50% glycine to a glycine-deficient basal diet containing 35% casein, arginine, cystine and the other usual components. In the 1st experiment the addition of ethanolamine increased the chick growth to the same extent as the

glycine supplement. At the same time other chicks with supplements of glycolic acid and glyoxylic acid had a depression in the growth rate. In the next experiment the above finding with ethanolamine was confirmed; in both instances the differences between the mean gains of the control and ethanolamine groups was statistically significant at the 1% level. Other groups of chicks in the 2nd experiment received a supplement of N-monomethylethanolamine, N-dimethylethanolamine, betaine or sarcosine. All of the latter substances failed to stimulate growth under the given experimental conditions. The significance of the above nutritional findings will be discussed in terms of metabolic pathways.

1728. DISTRIBUTION, CONCENTRATION AND PROPERTIES OF SALMONELLOSIS RESISTANCE FACTOR (SRF). Henry N. Wood* and Howard A. Schneider. Rockefeller Inst. for Med. Research, New York City.

The level of natural resistance of exogamous population of W-Swiss mice to fatal infection with Salmonella typhimurium can be raised by a nutritional factor (SRF). The quantitative estimation of SRF was conducted by means of the double strain inoculation method as modified by the use of genetically marked strains of S. typhimurium (J. Exper. Med. 103:207, 1956). SRF is found in several plant sources, including wheat and barley and in 1 animal source, commercial dried egg white. Testing of 27 varieties of wheat failed to reveal any pronounced differences in SRF referable to their genetic constitution. Malting resulted in increased SRF. SRF, which is destroyed by ashing, is extractable with methanol and soluble in water from which it is not removed by immiscible organic solvents, although it is dialyzable. SRF is adsorbed by charcoal and can be eluted with glacial acetic acid. Details will be presented of SRF subjected to column chromatography, paper chromatography, paper and starch block ionophoresis and Craig countercurrent distribution. Over-all concentration from wheat has been of the order of 1 millionfold with an efficiency of about 20%. One milligram of concentrate per 500 gm of diet results in a 90% protection from fatal salmonellosis.

1729. ENERGY VALUE OF DIET AND NITROGEN RETENTION. S. P. Yang* and Pearl Swanson. Home Economics Research, Iowa Agricultural Exp. Station, Ames.

Utilization of nitrogen by albino rats was investigated in relation to the caloric value of the ration when an amino acid mixture served as the sole source of dietary nitrogen. Experimental rations, force-fed 3 times daily, provided either 65, 50 or 35 calories/rat/day. In each instance, the daily quota of food furnished 74 mg of nitrogen, a quantity equivalent to that in 13 gm of a 4% lactalbumin ration. The amino acid mixture fed was one that has proved the most effective of several developed in our laboratory for the maintenance of body tissue at this level of nitrogen intake. Retentions were 80, 17 and -159 mg/7 days when the energy values of the diets, respectively, were 65, 50 and 35 calories/day. The varying caloric value of the diets was reflected in differences in the size and composition of the liver and in the amount and composition of carcass tissue. The data suggested that the number of calories needed daily when pure amino acids provided the dietary nitrogen depended on the methionine, cystine, phenylalanine and tyrosine contents of the mixture.

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Forty-first Annual Meeting

CHICAGO, ILLINOIS, APRIL 15-19, 1957

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1730. ACTION OF IMMUNE SERUM UPON A CELL-VIRUS COMPLEX. W. Wilbur Ackermann and Francis E. Payne,* Epidemiology Dept., School of Public Health, Univ. of Michigan, Ann Arbor.

A technique is described whereby the rates can be followed of various reactions involved in the initiation of infection of HeLa cells with poliovirus (type I Mahoney strain). The development of potential for cytopathology by the reacting fraction of cells is used to follow the progress of a particular reaction when that reaction is made rate-limiting. A single sequence of infection is achieved by the judicial use of antiviral immune serum, adding the serum after it can no longer influence the action of the viral inoculum and before the appearance of the viral progeny from affected cells. In measuring the development of cytopathology, advantage is taken of the inability of infected cells to remain attached to the glass substrate. The system is quantitated by the use of replicate cultures and direct counting of surviving cells. Two consecutive reactions have been recognized: the 1st results in a cell-virus complex stable to dilution and neutralizable by immune serum; the 2nd, which is relatively slow and obeys first order kinetics, represents the conversion of this complex into one resistant to the action of serum. This 2nd reaction will be considered in detail.

1731. IMMUNOCHEMICAL STUDIES ON PARTIALLY HYDROLYZED BLOOD GROUP A, B AND O (H) SUBSTANCES. Peter Z. Allen* and Elvin A. Kabat. Depts. of Microbiology and Neurology, Neurological Inst., Columbia Univ., New York City.

Mild acid hydrolysis of blood group substances at 100°C for 2 hr at pH 1.5-1.8 leaves a nondialyzable residue (P1 fraction) which shows decreased blood group activity and increased crossreactivity with type XIV antipneumococcal horse sera. Immunization of humans with P1 fractions obtained from purified A and B substances shows them to be antigenic. Moreover P1 fractions prepared from hog A substances were found to be antigenic in group A as well as in O individuals. Immunization of an A_1B person with P1 fraction from human B substance yielded antibody, shown by quantitative precipitin studies to be specific for the B P1 fraction since human and horse B preparations show little or no ability to precipitate unless subjected to mild acid hydrolysis. P1 fractions from horse A, hog A and O (H) preparations fail to crossreact. Bovine A, B and inactive preparations, however, do show crossreactivity regardless of blood group specificity or prior partial hydrolysis. The antiserum used was shown to be anti-P1 antibody by its specific ability to precipitate 70-106% of the hexosamine added as antigen. Capacity to precipitate antibody to B P1 appears to be unrelated to increased crossreactivity with type XIV antipneumococcal sera. Oligosaccharide inhibition studies show that B P1 specificity is distinct from B specificity although both involve a terminal nonreducing α-galactosyl residue; relative to α-methylgalactoside, melibiose is a poorer inhibitor in the B P1-anti B P1 system but a better inhibitor in the B-anti B system. (Supported by grants from the Office of Naval Research, the Natl. Science Fndn. and the William J. Matheson Commission.)

1732. INHIBITION OF IMMUNE HEMOLYSIS BY CONSTITUENTS OF SERUM. K. Amiraian,* O. J. Plescia, G. Cavallo* and M. Heidelberger. Inst. of Microbiology, Rutgers, The State Univ., New Brunswick, N. J.

Sheep serum, which has little or no hemolytic activity against sensitized sheep erythrocytes, inhibits immune hemolysis by complement (C') of guinea pig, pig and human sera, the only ones tested. Inhibition was found primarily in the water-soluble fraction obtained by dialysis. Sheep serum was also fractionated by zone electrophoresis on powdered cellulose. Several fractions among the α- and γ-globulins were found to be inhibitory. Pig serum, heated at 64°C for 30 min, inhibited hemolysis by guinea pig C' and human C', but not by pig C'. Here, too, inhibition was associated with the water-soluble fraction, which was more inhibitory than the whole heated serum and also inhibited pig C'. The lysis of partially complemented cells (EAC'1, 2, 4) supposedly requires only C'3. The reaction of EAC'1, 2, 4 with either guinea pig, pig or human serum was carried out in the presence of versene (10^{-2} M) to prevent the further action of C'1, C'2 or C'4. Contrary to expectation, the static titration curves for the 3 sera were not superimposable. This suggests either that some component other than C'3 is involved in the lysis of EAC'1, 2 4 or that inhibitory serum constituents play a part in this step. The latter will be discussed.

1733. USE OF PRECIPITATING ANTIBODY TO STUDY SALIVA OF SECRETORS AND NONSECRETORS OF INDIVIDUALS OF GROUPS A, B AND O. Harold Baer, Ingeborg Naylor* and Warner Kloepfer.*

Dept. of Microbiology, Tulane Univ. School of Medicine, New Orleans, La.

Chickens, immunized with purified blood group substances, respond with the production of precipitating antibody. Since this antibody forms a precipitate when mixed with whole saliva, family groups were studied by means of the quantitative precipitin test to follow the inheritance of the salivary antigens. For those families in which the parents' salivas precipitated well with anti-A, the children's salivas precipitated equally well, showing the classical pattern of inheritance both qualitatively and quantitatively. The antibody to the nonsecretor substance precipitated with those salivas obtained from individuals who did not secrete the A, B or O substance as determined by inhibition of hemagglutination. The anti-O antiserum precipitated well with salivas from individuals of group O but reacted with only an occasional sample from individuals of groups A and B. In one family, typed as group O, the salivas precipitated very poorly with all of the antisera.

1734. RELEASE OF HISTAMINE FROM RABBIT BLOOD BY MEANS OF ANTIGEN-ANTIBODY PRECIPITATES. J. F. Barbaro (intr. by E. L. Becker). Div. of Immunology, Walter Reed Army Inst. of Research, Washington, D. C.

Using the method for the determination of histamine developed by McIntire et al., it has been found that well washed specific precipitates prepared with rabbit antiserum released histamine when added to rabbit whole blood or platelets. On the other hand, specific precipitates made with sheep antiserum failed to release a measurable amount of histamine. The ability of the blood to release histamine varies markedly from rabbit to rabbit and even with the same rabbit from day to day; nevertheless, certain quantitative relationships are apparent. As little as 40-50 μg of precipitate nitrogen formed in the region of antibody excess or equivalence is capable of releasing substantial amounts of histamine. However, above a certain range of precipitate weight (240-500 μg of precipitate nitrogen) the release of histamine is not a function of the amount of precipitate, but rather of the ratio of antibody to antigen in the precipitate; in fact, within this range there was found a linear relationship between the ratio of antibody to antigen in the precipitate and the amount of histamine released. This relationship held whether the precipitate was prepared from heated (56° for 1/2 hr) or unheated antisera. Precipitates prepared from antiserum to which 0.01-0.02M ethylene-diaminetetraacetic acid had been added released 1.5 times more histamine than precipitates prepared from non-EDTA treated antiserum. The effect of other factors on the release of histamine will be described.

1735. BACTERIOPHAGE NEUTRALIZATION AS AN ASSAY OF PROPERDIN LEVELS IN FRESH SERUM. James L. Barlow,* Helen Van Vunakis* and Lawrence Levin. Div. of Labs. and Research, New York State Dept. of Health, Albany.

Fresh sera of various species neutralize E. coli T_2 phage. This property is dependent upon the properdin system. A method of assaying for vericidal activity in fresh serum has been developed. Reaction mixtures, containing constant phage (10^4 particles/ml) and increments of fresh sera in 0.126 M veronal buffer (pH 7.5) and 0.001 M Mg++, are incubated for 1 hr at 37°C. For control, the fresh serum is replaced by nutrient broth to determine the input virus and by the test serum heated at 56° C for 1/2 hr to check for the presence of heat-stable factors. The mixtures are diluted with chilled saline to stop the reaction and titrated for residual phage. One hundred human sera were assayed and only 3 possessed a heat-stable neutralizing factor. The data on the others fit the equation of von Krogh, between 25 and 75% neutralization. The slopes obtained from a logarithmic plot were found to be 0.35 ± 0.1. The phage neutralizing unit (PN_{50}) is defined as that quantity of serum which inactivates 50% of the phage under the above experimental conditions. The advantages and limitations of the assay as well as evidence with indicates that the PN_{50} reflect the properdin levels of serum will be discussed. Studies on the mechanism of virus neutralization by fresh sera and the inhibition exhibited by certain lipopolysaccharides in this system will be presented.

1736. EFFECT OF CALF SERUM ON CONCENTRATION OF POLIOVIRUS IN PREPARATIVE ULTRACENTRIFUGE. Samuel Baron (intr. by Joel Warren). Div. of Biologics Standards, Natl. Insts. of Health, Bethesda, Md.

In connection with the development of a poliovirus vaccine safety test, studies were undertaken to determine the efficiency of concentration of poliovirus from tissue culture fluids. Serum-free medium and serum-free experimental vaccines containing infectious virus were centrifuged in the no. 30 rotor of the Spinco model L preparative ultracentrifuge, and then separated into 2 supernatant fractions and pellet. Centrifugation for various intervals in repeated experiments resulted in recovery of the bulk of the virus in the lower supernatant fraction. This was taken to indicate sedimentation of virus to the base of the centrifuge cup but lack of adherence to the cup wall. Addition of calf serum results in formation of a firm visible pellet attached to the cup wall which includes most of the virus. The yield of virus in the calf serum pellet was found to depend on the duration of centrifugation and concentration of calf serum.

1737. DISSOCIATION BEHAVIOR OF HEMOCYANIN FROM THE GIANT KEY-HOLE LIMPET. Allen L. Bartel and Jerome R. Vinograd (intr. by Dan L. Campbell). Dept. of Chemistry, California Inst. of Technology, Pasadena.

Hemocyanin of the giant key-hole limpet, Megathura crenulata (Sowerby), has been extensively used in these laboratories as an intigen for general immunochemical studies. It is capable of easily producing high titer precipitin serums in rabbits and is one of the most readily available hemocyanins on the West Coast. From 1 to 2 gm can be obtained from a single limpet. The studies to be reported here consists of methods for purification by repeated centrifugation at 54,000 g and determination of physical and immunochemical properties. At pH 6.6 the final product shows a single molecular-weight component in the analytical centrifuge (Sw20 = 98). As the pH is increased, the hemocyanin reversibly dissociates into 2 components (Sw20 = 61 and 15) which vary in relative amounts until at pH 8.6 only the Sw20 = 15 component is present. Electrophoretically, at least 95% of the material shows only a single component over this pH range. The sedimentation behavior of the key-hole limpet hemocyanin is similar to other gastropod hemocyanins and unlike those from cephalopods and arthropods. This supports the idea that differences in sedimentation behavior of hemocyanins from different groups of animals reflect phylogenetic relationships. The hemocyanin gives several zones of precipitation (bands) when tested with specific rabbit antiserum by agar-diffusion techniques. An investigation is being made

of the relationship of these multiple zones of precipitation to the dissociation products of hemocyanin and will be discussed.

1738. TOXICITY OF GUINEA PIG SERUM FOR GUINEA PIGS. Jack R. Battisto (intr. by Merrill W. Chase). Dept. of Microbiology, Univ. of Arkansas School of Medicine, Little Rock.

On the basis of dermal reactivity to the primary inoculation of normal guinea pig sera, an inbred strain of normal guinea pigs may be separated into 3 distinct groups. While studying passive cutaneous anaphylaxis in guinea pigs, a few animals were observed to react to dermally deposited guinea pig serum. Cutaneous reactions were characterized as erythematous, slightly edematous or maximal at 18-24 hr. The animals, an albino pen-inbred strain utilized at the Rockefeller Institute for Medical Research, were fed and housed in the same way. Following the initial observation, fresh guinea pigs were bled from the heart and clear serum rendered from each clot in the usual manner. The individual undiluted sera were injected intradermally in 0.1-ml amounts so that an individual received its own serum plus samples of serum from other guinea pigs. In several such experiments, totalling 126 animals from 4 generations, it was seen that all guinea pigs reacted minimally (5 mm or less) to their own serum. Minimal reactions to all sera uniformly were observed in 66% of the animals. Maximal reactivity (20 mm) to sera of certain low reactors was manifested by 20% of the group. The remaining 14% displayed intermediate reactions (10 mm) to the same sera. A majority of the minimally reacting group possessed sera capable of inducing the reactions described. Hemagglutination tests, using washed red blood cells of high reactors plus serum of low reactors, and vice versa, failed to indicate the presence of blood groups. Precipitin and neutralization tests with the sera of the 2 most dissimilar groups did not detect circulating antibody. Animals younger than 70 days did not respond maximally. It was not possible to increase the reactivity of animals older than 70 days by means of repeated cutaneous injections of sera over a period of several weeks. (Aided by the Natl. Insts. of Health Research Grant E-1364.)

1739. MIXED INFECTIONS BETWEEN ECHO AND POLIOVIRUSES. Matilda Benyesh,* Heihachi Itoh,* G.-D. Hsiung* and Joseph L. Melnick. Section of Preventive Medicine, Yale Univ., New Haven, Conn.

Because echo and polioviruses have many properties in common and yet have distinguishing markers, experiments were designed to determine whether recombination might occur between them. Monkey kidney cells were infected simultaneously with equal amounts of polio-1 (Mahoney) and echo-1 (Farouk) viruses in high concentration, and the progeny plated in limiting dilution. Plaques were picked on the 7th-9th days and their progeny, obtained by passage into a tube culture containing 10^5 cells, were typed by the pH panel test. One clone, of 129 picked from the 1st passage, was not neutralized by either parent serum but was neutralized by the mixture of both. The plaque method showed that the clone was made up of a mixture of particles, 52% of which were neutralized by Mahoney and 48% by Farouk antiserum. The other clones were neutralized by either one or the other of the parent antisera. The progeny of the clones with mixed particles were plated in limiting dilution, the clones passed and their progeny typed. In the 2nd passage, 171 plaques were tested, and 10 yielded mixed progeny. By the plaque neutralization test, varying proportions of echo and poliovirus particles were found among them. In addition, 25 other clones gave evidence of doubly neutralizable particles in the panel neutralization test. A 3rd passage at limiting dilution yielded clones which gave more reactions of the latter type (73 of 179 plaques tested), but none of those tested have yet been proven to be doubly neutralizable by the plaque technique.

1740. RELATION BETWEEN PHYSICAL PROPERTIES OF ADJUVANT VACCINES MADE WITH PETROLEUM, SYNTHETIC, OR VEGETABLE OILS, AND ANTIBODY RESPONSE. Byron S. Berlin (intr. by Thomas Francis, Jr.). Epidemiology Dept., School of Public Health, Univ. of Michigan, Ann Arbor.

Physical properties of influenza virus vaccines emulsified in a variety of oils were correlated with antibody response to these vaccines. Antibody response in albino mice to either aqueous vaccines or water-in-oil vaccines was determined by hemagglutination-inhibition with sera obtained 6 wk after inoculation. Stability of emulsified vaccines, determined by storage at 37°C, was found to be a requisite for adjuvant activity. Stable influenza virus vaccines emulsified in petroleum oils can be made over a wide range of either water-to-oil ratios or viscosities. Emulsion-vaccines made in the mid-range of water-to-oil ratios were found to induce better antibody response than vaccines made at either extreme of the range. Moderately viscous petroleum oil emulsion-vaccines were found superior to highly viscous vaccines. A synthetic oil, liquid polyethylene, could be substituted for mineral oil, yielding stable, moderately viscous emulsion-vaccines which induced an adjuvant response. Vegetable oil emulsion-vaccines were found to possess poor stability and did not induce an adjuvant response. Evidence obtained by study of vaccines emulsified in mineral oil led to experiments demonstrating that dispersion of a lyophilic solid (Thixcin) in vegetable oil prior to emulsification enabled formation of stable vegetable oil emulsions. Emulsified vaccines made with castor oil containing Thixcin were highly viscous and induced an adjuvant response. Antibody responses to the new emulsified vaccines determined at 6 wk compare favorably with responses to conventional emulsified vaccines.

1741. CHEMICAL NATURE AND BLOOD GROUP SUBSTANCE CONTENT AND ACTIVITY OF ASTHMATIC SPUTUM. Aline W. Berns* and Samuel C. Bukantz. Dept. of Medicine, Washington Univ., St. Louis, Mo.

Chemical studies have been performed on ethanol precipitates of 75 viscous sputa derived from 53 asthmatic subjects. The following range of values was obtained: total carbohydrate (as glucose) 6.2-28.2%, nitrogen 9.0-13.0%, hexosamine 11.9-15.0%, fucose 4.5-5.5%, and total solids 13.3-33.8 mg/ml. Two-dimensional paper chromatograms of acid hydrolysates of the individual precipitates yielded amino-acid patterns like that of most proteins with slightly larger quantities of threonine and phenylalanine. One-dimensional chromatograms for carbohydrates yielded galactose, mannose, fucose and hexosamine. Pooled ethanol precipitates were extracted with phenol after pepsin digestion and yielded a highly viscous material in both phenol soluble and phenol insoluble fractions, both of which were extremely active in inhibiting hemagglutination. Chemically these isolated fractions closely resembled purified blood group substance but were immobile on paper electrophoresis in either

veronal or borate buffers while commercial purified blood group substances of animal origin migrated towards the cathode in veronal and towards the anode in borate buffer. The data suggested that the mucopolysaccharides of bronchial mucus might exhibit cross-reactivity in the ABO system. Initial sutdies have been made with 35 individual sputa derived from asthmatic subjects of the following ABO distribution: 13-A (37%); 18-0 (51.6%); and 4-B (11.4%). These have usually inhibited hemagglutination specifically but several B sputa strongly inhibited both A and B, and several O sputa inhibited A agglutination. (Supported by Grant H-1752 from the Public Health Service and Armour Labs., Kankakee, Ill.)

1742. RESPONSE OF RABBITS TO INJECTIONS OF LOW MOLECULAR WEIGHT CARBOHYDRATES. Nancy J. Bigley,* Matthew C. Dodd and Francis W. McCoy.* Depts. of Bacteriology and Medicine, Ohio State Univ., Columbus.

Rabbits were injected intramuscularly with 0.25 to 5.0 gm alpha-d-galacturonic acid and 1.0-2.0 gm of glucurone incorporated in adjuvants. Twelve to 28 days later all rabbits developed reactions at the site of injection varying from small nodules to massive swelling of the area, depending on dosage. About the same time skin tests performed with the substances became positive. These were essentially delayed type reactions, although the hypersensitivity was transferred to normal rabbits with serum. The skin tests were typified by areas of erythema, reaching maximum usually in 24 hr at which time the central portions were pale and hard. The diameter of the skin tests varied from 1 to 2 in. Reactions occasionally were most marked at 48 hr and remained fully developed as long as 96 hr. No in vitro antibody demonstration was obtained with serum although it was detectable at times in eluates from red cells of some rabbits. Electrophoretic patterns of these sera were abnormal and varied over a period of several weeks. Several animals receiving the larger doses demonstrated loss of weight, irritability and weakness in 4-8 wk and were killed. The kidneys, liver, spleen and lungs were grossly abnormal. Histologic examinations of the tissues of these animals indicated hypersensitivity with fibrinoid changes, vasculitis and endothelial proliferation, some of which were similar in certain respects to changes described as characteristic of the collagen diseases.

1743. MEASLES VIRUS GROWN IN TISSUE CULTURE. Francis L. Black (intr. by Joseph L. Melnick). Section of Preventive Medicine, Yale Univ., New Haven, Conn.

Many heretofore unresolvable characteristics of measles virus and immune reactions of the host have become available for study since the isolation of measles in tissue culture by Enders and Peebles. The studies reported here were made in a human cancer cell strain, Hep-2. Filtration experiments through gradocol membranes indicated the virus to be in the medium size range, 100-200 mμ. The volume sensitive to ionizing radiations is considerably smaller, indicating that only a portion of the virus is irreplaceably essential for reproduction. The size of the complement-fixing antigen, as determined by irradiation, was smaller yet. When tissue cultures, grown in complete medium, were massively infected, the first new virus was detectable after 18 hr if the cells were disrupted by freezing, but it was not naturally released into the fluid until 30 hr after inoculation. C-F antigen was first detected some time after infectivity was recognizable but the delay may have been due to the difference in sensitivity of the methods. Both C-F and neutralization tests may be conveniently carried out and tests have been made on sera from humans of different ages and from a variety of African and Asian simian species.

1744. EFFECTS OF ANTI-MOUSE SPLEEN ANTIBODIES. Daniel A. Boroff and Richard A. Zucker.* New England Inst. for Med. Research, Ridgefield, Conn.

Antibodies, produced by injecting rabbits with saline extract of mouse spleens, when administered to mice by the intramuscular route had a singular effect. Mice, which received 0.3 ml of this serum in 0.1-ml doses on 3 consecutive days, became apathetic, their fur presented a roughened appearance and they lost weight. On autopsy, these mice showed a characteristic enlarged dark-red spleens which were 2-3 times the normal size. Histologically, these spleens showed a large number of clear cells with one or more nuclei. There was moderate edema, increased activity in the follicles and a large amount of blood in the sinusoids. The peripheral blood obtained from the tail vein 4 hr after the serum injection showed greatly decreased numbers of platelets and some leukopenia. Differential counts, 24 hr after the 3rd injection, showed numerous young neutrophils, large clear mononucleated cells resembling those seen in infectious mononucleosis and platelets in large clumps. Such mice were more susceptible to experimental infection with Klebsiella penumoniae than mice injected with normal rabbit serum.

1745. PHYSICAL STUDIES OF ADENOVIRUS. F. B. Brandon,* A. R. Taylor, M. Patterson* and I. W. McLean, Jr. Research Labs., Parke, Davis and Co., Detroit, Mich.

The purpose of the work to be described was to study the physical characteristics of particulate material found in preparations of adenovirus propagated in H.E. p.#2, and monkey kidney cell cultures. Type III adenovirus was the prototype used for all experiments. The biological properties of interest found in crude suspensions, depending on the passage history of the virus, included cytopathogenicity, hemagglutinating activity and complement-fixing activity. Preliminary physical studies indicated that a partial separation and concentration of these properties could be achieved by filtration and differential centrifugation of infectious material. Four distinct kinds of figures were revealed in electron micrographs of the concentrated preparations. These included 5-sided particles with a suggestion of internal structure, long slender filaments or hairs and 2 classes of round particles easily distinguished by their size. The relative and absolute sizes of the particles will be discussed, and the results of further physical studies on concentrated purified suspensions will be shown. Qualitative evidence for the association of certain biological properties with the particles of interest will be presented.

1746. ANTIGENIC MUTANTS OF INFLUENZA VIRUS. B. A. Briody. Virus Lab., Dept. of Microbiology, Hahnemann Med. College, Philadelphia, Pa.

The method employed to isolate antigenic mutants of influenze virus is based on earlier studies with beta-inhibitor (Virology 1:301, 195). When selective pressure in the form of antibody is exerted during the course of 1 egg passage on the BH (1935) strain of influenza virus, antigenic mutants may be detected at a rate of about 1 mutant particle to 10^8 parent virus particles. On the basis of neutralization and hemagglutinin-inhibition tests, the mutants have been much more closely related antigenically than the parent virus to

strains of influenza virus isolated since 1935. In addition to the change in antigenic structure the mutants have concomitantly shown a greater sensitivity to beta inhibitor, a greater stability of the hemagglutinin to inactivation by heat and a loss of convertibility to the indicator state (sensitivity to alpha-inhibitor). All of these changes might conceivably be due to one chemical change in the surface configuration of the virus particle. When similar studies were carried out with other strains of influenza virus (FM1 - 1947, Cuppett-1950) and Sweden - 1950), the virus particles which survived and eventually grew out were in every instance antigenically indistinguishable from the parent virus. Serial passage of these strains of influenza virus in the presence of specific immune serum has permitted the isolation of antigenic mutants of each of the parent strains. The latter data tend to indicate that strains of influenza virus mutate antigenically at widely different rates.

1747. SEROLOGIC RESPONSE OF SCHOOL CHILDREN TO DELAYED SECOND INOCULATION OF POLIOMYELITIS VACCINE. Gordon C. Brown. Epidemiology Dept., School of Public Health, Univ. of Michigan, Ann Arbor.

Irregularities in the administration of poliomyelitis vaccine have raised concern over its efficacy under conditions at variance with recommended inoculation schedules. One such variation encountered frequently has been a delay of several months between 1st and 2nd injections. The opportunity to study serologic responses to delayed 2nd inoculation was presented when Ann Arbor school children of the first, second and third grades received their 1st injection in April, 1955 and their 2nd, the following September. Blood specimens were obtained from 221 subjects on the day of the 2nd injection and again 2 wk later. A small group of 10 children receiving their 1st inoculation at the same time served as controls. Neutralization tests with paired sera were performed in HeLa cell tissue cultures against 3 types of poliomyelitis virus. Thirteen per cent of the children had no demonstrable antibody to any type of virus before the secondary stimulus but none were left without some antibody following the 2nd injection. In addition, the percentages of children without antibodies to specific types were drastically reduced following the 2nd inoculation and the percentage with antibodies to all 3 types was increased from 33 to 85. Furthermore, the geometric mean of the antibody titers for each type showed a substantial increase as a result of the secondary stimulus. These results compared favorably with studies on children who had received the first 2 doses of vaccine at an interval of 1 mo thus indicating no immunological disadvantage in delaying the 2nd inoculation.

1748. CROSS REACTIONS BETWEEN HEN AND DUCK EGG ALBUMINS. Dorothy J. Buchanan* and J. Oudin. Institut Pasteur, Laboratoire d'Immunochimie analytique, Paris, France.

A rabbit anti-hen egg albumin serum, nonabsorbed or absorbed with varying amounts of duck egg albumin (DEA) up to the point of maximum antibody nitrogen precipitation, was used. In the agar diffusion method (technique of simple diffusion in tubes), either hen egg albumin (HEA) or DEA alone gave the expected straight line with nonabsorbed antiserum when the penetration was plotted against the logarithm of the antigen concentration or the antiserum concentration. When the 2 antigens were mixed in varying proportions using nonabsorbed antiserum, the penetrations of HEA remained a straight line function of the logarithm of the antigen concentration with a slight increase in penetration. The penetrations of DEA plotted against the logarithm of the antigen concentration were almost a straight line function when the concentration of HEA was low but assumed a curved shape and sometimes the zone precipitation of DEA disappeared with higher concentrations of HEA and lower concentrations of DEA. Partial absorption of antiserum with DEA caused less interaction between the antigens and increased the penetrations of both antigens. No interaction could be observed in antiserum completely absorbed by DEA. The effect of the DEA concentration on the penetration of HEA was most pronounced when the concentration of HEA was low and that of DEA high and on both the non-absorbed antiserum and that absorbed with the lower amounts of DEA. HEA influenced the penetration of DEA less when HEA was present at lower concentrations and the antiserum was absorbed with larger amounts of DEA. The antibody nitrogen determined by the agar diffusion method corresponded with the chemical quantitative nitrogen precipitations when HEA was the antigen. The results with the heterologous DEA were much higher by the agar diffusion method than by the chemical determinations. The quantitative work suggested the possible formation of soluble complexes with DEA which did not precipitate but which were capable of influencing the penetration of the antigens in the agar.

1749. ANTIGENICITY OF ASTHMATIC SPUTUM: ABO HEMAGGLUTINATING AND GEL-PRECIPITATING ACTIVITY OF RABBIT ANTI-A-SPUTUM SERUMS. Samuel C. Bukantz and Aline W. Berns.* Dept. of Medicine, Washington Univ., St. Louis, Mo.

As part of a series of investigations on the chemical nature of bronchial mucus, rabbits were immunized with a sterile suspension of viscous sputum from an asthmatic subject of blood group A. Anaphylaxis was encountered early and surviving animals were bled after having received 17.0 mg of sputum solids in 10 i.v. injections. Antisera from these animals (RASA) agglutinated A erythrocytes to high titer, B and O to a lesser degree, and produced multiple bands of precipitation in agar gels when diffused against sputa derived from O, A and B asthmatic subjects. Several common as well as specific antigens were apparently present. RASA produced no bands with 8 individual human serums or soluble extracts of bacteria common to the bronchial flora. Lysates of A, B and O erythrocytes yielded bands with RASA and reactions of identity when paired with sputa of O, A or B subjects although specific reactions remained for each lysate. After absorption of ABO agglutinins from RASA with corresponding erythrocytes, precipitins for lysates and sputa were of diminished intensity. Arthus skin reactions were elicited in the immunized rabbits tested with hog and horse blood group substances and homologous sputum antigens. Rabbits immunized with sputum suspended in Freund's adjuvants failed to develop agglutinins or positive skin tests. The observations encourage the belief that immunological methods will be useful in ascertaining the origin, chemical structure and mechanism of intrabronchial accumulation of the bronchial mucus which characterizes certain forms of asthma. (Supported by grants from Armour Labs. and Public Health Service (H-1752).)

1750. INHIBITION OF IMMUNE HEMOLYSIS BY ITS PRODUCTS. G. Cavallo,* O. J. Plescia, K. Amiraian* and M. Heidelberger. Inst. of Microbiology, Rutgers, The State Univ., New Brunswick, N. J.

Guinea pig complement (g. p. C') and pig complement (pig C') were strongly inhibited by the lysate from the reaction of sensitized sheep erythrocytes with pig C'.

The inhibition was associated with the stroma and its rate varied directly with the concentration of stroma and the temperature. Both g. p. C' and pig C' were incubated at 37°C with concentrations of stroma equivalent to those found as products in immune hemolysis; the stroma were separated by centrifugation and the supernates were titrated for C' and the components of C'. In each case there was a loss in total activity and marked decreases in the titers of C'2 and C'4 (2nd and 4th components of C'). Stroma of cells lysed by g. p. C' also inhibited pig C' but inhibited g. p. C' only slightly. The reason for this behavior is not clear although there were differences in the appearance of the stroma depending upon the C' used for lysis of the cells. The stroma of cells lysed by pig C' sedimented more completely and were more compact and opaque. Pig C' was also inhibited by stroma derived from both sensitized and nonsensitized erythrocytes lysed osmotically. However, g. p. C' could be inhibited only by stroma of sensitized erythrocytes. Pig serum is known to have natural hemolysins against sheep erythrocytes and so can explain the difference in behavior between pig C' and g. p. C'. Inhibition of immune hemolysis by stroma and peptides as similar in that both inhibited C'2 predominantly. (cf. Plescia et al. Fed. Proc., this issue). Since stroma contain much protein, they could furnish substrates for proteolytic components of C' and so inhibit immune hemolysis competitively.

1751. STUDY OF THE POSSIBLE RELATIONSHIPS OF PHAGOCYTOSIS TO VIRULENCE AND IMMUNITY IN PLAGUE. D. C. Cavanaugh, M. L. Spivack and R. Randall (intr. by M. R. Hilleman). Dept. of Hazardous Operations, Walter Reed Army Inst. of Research, Washington, D. C.

In a recent series of papers, Burrows and coworkers of England have described some differences between virulent and avirulent plague bacilli in their ability to resist phagocytosis. They reported that, after a short period in the animal body, virulent bacilli became highly resistant to phagocytosis while avirulent bacilli remained susceptible. Further work demonstrated that this characteristic could also be produced in vitro by growing virulent organisms in a rich, complex medium (Burrows and Bacon. Brit. J. Exper. Path. 35:129, 1954; 37:481, 1956). Studies were undertaken in this laboratory in an attempt to ascertain the importance of the above-mentioned phenomena in respect to the immune animal. Mice were immunized with 1 intraperitoneal dose of 10^5 living, avirulent Pasteurella pestis strain EV_{76}. The animals were used approximately 2 wk following immunization. The virulent strain 195/P was used as the test organism. Comparisons of the phagocytosis of resistant plague bacilli grown in vitro by normal and immune mice were made and no appreciable differences in the rate of phagocytosis were noted in either group. No differences in the number of resistant or sensitive virulent organisms required to produce fatal bubonic or pneumonic plague infection in immunized mice were observed. Convalescent sera obtained from guinea pigs and antisera produced in rabbits against living, virulent bacilli were used to sensitive both sensitive and resistant bacilli. Resistant bacilli were produced in vivo and in vitro. No opsinization was observed with resistant bacilli grown in vitro. Resistant bacilli grown in vivo were opsinized by both rabbit and guinea pig antisera. The significance of these findings will be discussed.

1752. INHIBITION OF RHO (D) ANTIBODY BY ELUATES FROM RHO (D) ERYTHROCYTES TREATED WITH MUMPS VIRUS. Robert W. Chandler,* Nancy J. Bigley* and Matthew C. Dodd. Dept. of Bacteriology, Ohio State Univ., Columbus.

It has been demonstrated in this laboratory by Bigley that an inhibitor of Rho (D) antibody may be detected in the eluate of Rho (D) positive erythrocytes treated with mumps virus or the periodate ion. This phenomenon was found to be specific with respect to the virus and the antigenic type of red cells employed. This investigation is a study of the properties of this inhibitor and the various factors influencing the reaction, such as cell-virus ratio, pH and inhibitor stability. Of particular interest was the wide fluctuation of the inhibitor titer over a period of 3-4 hr treatment with mumps virus. Experiments indicated that the virus particle and the inhibitor were being eluted simultaneously from the cell surface. Furthermore, it was noted that a correlation existed between the decrease of the inhibitor titer and the viral hemagglutination titer. The elimination of active virus by antimumps serum or heat resulted in a stable inhibitor titer. The tentative identification of inhibitor as Rho (D) antigen, its release from cells by both agents and the possible mechanism of the inhibitor titer fluctuations will be discussed as well as the relation of virus receptor to the Rho (D) antigen.

1753. PREPARATION AND FORMALDEHYDE INACTIVATION OF PURE POLIOVIRUS. Jesse Charney, W. P. Fisher and Roy A. Machlowitz (intr. by Alfred A. Tytell). Dept. of Cytochemistry, Merck Sharp and Dohme Research Labs., West Point, Pa.

Poliovirus was concentrated and purified by a modification of the method of Schaffer and Schwerdt. End-point dilution titrations, plaque counts and complement fixation assays were used to measure the virus concentrations throughout the purification procedure. Within the limits of precision of the various assay methods, a satisfactory correlation of the 3 measures was obtained from crude tissue culture fluid to the final pure virus. Pure poliovirus at concentrations approximately 100 times as great as that in tissue culture filtrates was inactivated with formaldehyde (92.5 γ/ml). The reaction seemed to be approximately first order and complete extinction of infectivity took place in 42-48 hr. Complement fixation assays were run at various times during the inactivation procedure up to 7 days; under certain conditions the complement fixation titer could be maintained throughout the period of inactivation. The influence of a number of conditions upon the inactivation of poliovirus with maintenance of complement fixation titers will be discussed.

1754. INCORPORATION OF S^{35}-AMINO ACIDS INTO ANTIBODIES IN PRIMARY AND SECONDARY RESPONSE. Hsieh Fu Cheng* and Felix Haurowitz. Chemistry Dept., Indiana Univ., Bloomington.

Rabbits were immunized by 6 injections of 50 mg of ovalbumin (OA) given over 3 wk. A single dose of hydrolyzed S^{35}-yeast protein was injected on the 7th, 17th, 27th or 37th day after the final injection of OA (=OA-I-6), and a challenging dose of OA (=OA-II) on the 34th day after injecting OA-I-6. Serum samples were taken shortly before and 1 and 2 days after inactivated at 55° and found to be free of precipitating antibody. All these sera contained S^{35}-proteins (co-precipitating antibodies ?) which were specifically adsorbed to precipitates of OA and anti-OA, but not to those of BGG and anti-BGG (BGG = bovine gamma-globulin). Sera obtained 5 days after injecting OA-II contained precipitating antibodies. The specific activity of the antibody protein, in counts/min/mg, was 70-95% of that of the total globulins of the same sera, even if the S^{35}-amino acids had been injected 27 days

before OA-II. It is difficult to reconcile the high S^{35}-activity of the antibodies with the current view that all of them are formed after the challenging injection (OA-II). Our results indicate that a considerable portion of the antibodies was formed prior to the injection of OA-II and stored in the tissues; release into the blood serum occurs during the secondary response. (Supported by the Natl. Science Fndn. and the Public Health Service, and by contracts of Indiana Univ. with the Office of Naval Research and the Atomic Energy Comm.)

1755. CULTIVATION AND TITRATION OF VACCINIA IN BOVINE, AVIAN AND PRIMATE CELL TISSUE CULTURES. Ernest Cutchins* and Joel Warren. Div. of Biologics Standards, Natl. Insts. of Health, Bethesda, Md.

This investigation was undertaken to explore the application of tissue culture techniques to the assay of smallpox vaccines and the possible use of serial passage bovine cells as a source of this biological product. Vaccinia virus, derived from commercial bovine lymph vaccine, was serially propagated in cultures of bovine muscle, kidney and lung and monkey kidney. The adapted virus strains were then titrated in homologous and heterologous cells, in embryonated eggs and on the skin of rabbits to determine differential susceptibility. Vaccinia virus from bovine lymph was also included in this comparison. Virus yields from several types of tissue cultures were also determined. Vaccinia virus from any source titered 1-2 log dilutions higher in monkey kidney cultures than in bovine cells and this primate cell appears comparable to the rabbit skin in its susceptibility. Four commercial lots of smallpox vaccine had titers of 1×10^8 or greater $TCID_{50}$/ml when titered in monkey kidney as compared with an average titer of 1×10^6 in bovine cells. However, bovine tissue cultures gave reproducible endpoints and were less difficult to maintain for the long incubation periods required than any other type of cell studied. Comparison of virus yields (using vaccinia adapted to the host cell by 10-13 serial passages) revealed that both monkey kidney and bovine cell cultures produced the same quantities of virus, approximately 1×10^7 $TCID_{50}$/ml. These findings will be amplified and their bearing on production and testing of smallpox vaccines discussed.

1756. METABOLISM OF ARTERIAL TISSUES OF RATS WITH EXPERIMENTAL HYPERTENSION. Marie M. Daly and Esmeralda Gurpide (intr. by David Seegal). Columbia Univ. Research Div., Goldwater Mem. Hosp., and Depts. of Biochemistry and Medicine, Columbia Univ., New York City.

Certain aspects of the oxidative metabolism of arterial tissues of normal rats and of rats made hypertensive with desoxycorticosterone acetate and NaCl were compared. The preparation studied consisted of intima and media. Cytochrome oxidase activities of aorta and of the larger peripheral arteries (brachial and iliac) were determined, and the oxygen consumption of isolated aorta was also measured. Treatment of the data was based on the nonelastin noncollagen nitrogen content of the tissue and on the desoxypentosenucleic acid content as well as on tissue weight and total nitrogen content. In one experiment aortas of 12 hypertensive rats showed average increases of 65% in cytochrome oxidase activity and of 40% in oxygen consumption per unit of tissue weight or of total nitrogen when compared with 12 normotensive controls. On the other hand, when the measurements were compared on the basis of nonelastin noncollagen nitrogen content or of desoxypentose nucelic acid content, the increase was only 20%. The changes in the peripheral arteries were similar to those in aorta but less pronounced. The change in metabolic activity seems to be largely a consequence of hypertrophy of the vessels, which involves an increase in their content of smooth muscle.

1757. AN INVESTIGATION OF THE "L" CELL/HERPES VIRUS INTERACTION IN SUBMERGED (SPINNER) CULTURE. Eldon V. Davis,* William F. McLimans, F. L. Glover,* C. J. Kucera,* D. W. Ziegler* and Geoffrey W. Rake. Wistar Inst. of Anatomy and Biology, and Univ. of Pennsylvania, Philadelphia.

An investigation was made of the host cell/virus interaction with the L cell propagated in submerged culture of the "spinner type." The L cells were experimentally infected with Herpes simplex virus. At frequent intervals thereafter small aliquots of the culture were removed for the purpose of determining: the host cell population, both by direct and nuclei count; the rate of replication of infectious virus; the cytologic changes including time of occurrence of typical inclusion bodies, as well as the mitotic state of the host cell. A typical virus growth curve was observed. Cellular necrosis as a result of viral replication occurred although approximately 20% of the cells exposed to virus survived and were subsequently propagated in both the spinner and conventional bottle culture. Data relating to nuclei count and direct cell count suggested an early fragility of the infected host cell nucleus. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis.)

1758. VITAMIN B_{12} BINDING AND PAPER ELECTROPHORETIC PATTERNS OF SERUM. Robert L. Davis* and Bacon F. Chow. Research Lab., VA Hosp. and Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.

Serum samples obtained from normal and diseased human adults were studied for vitamin B_{12} binding capacity (L. leichmannii ATCC 4797-resting cell technique) and protein patterns by paper electrophoresis (Methods of Durrum and Kunkel). This report summarizes our findings with particular reference to tuberculous patients and pregnant women with high serum vitamin B_{12} binding capacities. The results indicate a correlation between high serum vitamin B_{12} binding levels (greater than 1.8 millimicrograms of an added 2.0 mμg of vitamin B_{12} combined with 0.5 ml serum) and elevations of alpha 1, 2 globulins. When tuberculous patients were treated with chemotherapeutic agents their improvement in clinical status was accompanied by a marked reduction in the vitamin B_{12} binding levels and an approach to normal paper electrophoretic patterns. Pregnant women with elevated vitamin B_{12} binding levels likewise had abnormal protein electrophoretic patterns with elevated alpha and markedly elevated beta globulins. In approximately 10% of the serum samples obtained from tuberculous patients, a high binding capacity was not accompanied by a corresponding elevation in the alpha globulins. Since vitamin B_{12} binding substances other than serum proteins may be liberated into the blood during certain stages of disease or pregnancy, deviations from a set pattern may result. Several interrelationships among certain blood constituents such as C-reactive protein, nonglucosamine, polysaccharides and others with the vitamin B_{12} binding and protein electrophoretic patterns will also be reviewed.

1759. SYNTHESIS OF SERUM PROTEINS IN THE NEONATAL AND DEVELOPING RABBIT. Maria

P. Deichmiller* and Frank J. Dixon. Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The pattern of serum protein syntheses by neonatal and developing rabbits was studied by measuring the uptake of S^{35}-labeled amino acids into the various serum proteins. S^{35}-labeled amino acids in the form of yeast cell hydrolysates were injected i.m. into the animals. One day later serum was obtained and separated by starch block electrophoresis. The various protein fractions were eluted from the block and analyzed for nitrogen content and S^{35} specific activity. All calculations were corrected to a constant injection of S^{35}/unit body weight. During the first week of life the beta globulin concentrations are above normal adult levels, alpha globulin values are comparable to adult values and albumin and gammo blobulin values are low. At this time incorporation of S^{35} into alpha globulins occurs at rates comparable to those in the normal adult but there is little incorporation into albumin, beta globulin and gamma globulin. At 1 mo of age the concentrations of albumin, alpha globulin and beta globulin approach normal adult values while gamma globulin levels remain low. At this time S^{35} incorporation into all protein fractions is comparable and approaches adult values. At 50 days a readily demonstrable concentration of gamma globulin is evident in the serum. (Supported by Atomic Energy Comm. Contract No. AT (30-T) 1205.)

1760. INTRACELLULAR LOCALIZATION OF ADENOVIRUSES. Floyd W. Denny, Jr.,* Georgiana S. Boyer* and Harold S. Ginsberg. Dept. of Preventive Medicine, WRU School of Medicine, Cleveland, Ohio.

Electron microscopy of HeLa cells infected with adenoviruses has shown virus-like particles, principally in the nucleus, but occasionally in much smaller numbers in the cytoplasm (Kjellén; Harford; Morgan). To determine the site of development of infectious virus, cytoplasmic and nuclear fractions of infected HeLa cells were prepared by mechanical disruption and differential centrifugation in 0.25 M or 0.88 M sucrose solutions. In experiments done with type 4 adenovirus the cytoplasmic fractions contained the majority of virus present in the whole cell: 40 to 100+% (average, 78%) in the cytoplasmic fraction, and <1 to 13% (average, 7%) in the nuclear fraction. Inclusion in the cytoplasmic fraction of virus from a variable number of cells totally disrupted by the blender complicates the interpretation of these results. In all experiments, however, more virus was associated with the cytoplasmic fraction than could be accounted for by the percentage of cells destroyed; no evidence was obtained of excessive selective destruction of infected cells. That infectious virus was also located in the nucleus was suggested by the release of virus by disruption of nuclei. Similar data were obtained with cultures studied 22-120 hr after infection. Studies with type 1 adenovirus confirmed these results.

1761. ANTIBODY PRODUCTION BY CELLS OF THE NEONATAL RABBIT.† Frank J. Dixon and William O. Weigle.* Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The inability of the neonatal rabbit to make antibody is well recognized. Recently it has been observed that adult rabbit lymphoid cells capable of antibody synthesis after their transfer to X-irradiated adult rabbits do not make antibody after their transfer to neonatal recipients. This suggests that it is the internal environment of the neonatal rabbit and not necessarily the lack of cells potentially capable of antibody production that is responsible for its immunological inadequacy. To test this possibility spenic and thymic cells of neonatal rabbits were exposed to shigella toxin in vitro, washed and transferred to neonatal and X-irradiated adult recipients. No significant agglutinin responses were observed in 14 neonatal recipients of live exposed cells or in 6 adult, X-irradiated recipients of heat killed exposed cells. However, in 9 of 20 adult X-irradiated recipients of live, exposed neonatal cells, significant agglutinin titers developed. This would suggest that lymphoid cells of the neonatal rabbit can, under certain conditions, make an antibody response. Attempts were also made to alter the internal environment of the neonatal rabbit by injecting into them normal adult lymphoid cells prior to the transfer of adult lymphoid cells sensitized to Shigella toxin. Significant antibody responses were observed in 16 or 38 neonatal recipients pretreated with normal lymphoid cells and prior to the injection of sensitized lymphoid cells and 0 of 23 neonatal recipients injected with sensitized lymphoid cells only. It appears from these data that injection of the normal adult lymphoid cells can make neonatal rabbits adequate recipients of antibody producing cells. (Supported by Atomic Energy Comm. Contract No. AT (30-1-1205.)

1762. ENHANCEMENT OF MOUSE SPLENIC DESOXYRIBONUCLEASE ACTIVITY BY INTRAVENOUS ADMINISTRATION OF CRYSTALLINE OR CELLULAR DESOXYRIBONUCLEIC ACID. David M. Donaldson and Frank D. Tidwell,* Dept. of Bacteriology, Brigham Young Univ., Provo, Utah.

Following the ingestion of the nucleated chicken erythrocyte (crbc) by either the peritoneal or the splenic phagocyte, one of the first observable morphological changes is the disappearance of the crbc chromatin material. In vitro studies with peritoneal phagocytes revealed that this intracellular digestion of crbc nuclear material was increased following immunization with crbc's and was not dependent on circulating hemolysin consentrations (J. Immunol. 76:192, 1956). Enzyme studies were initiated in an attempt to determine why previous exposure to crbc's increased intracellular digestion of the erythrocytes nuclear material. Within 24 hr after a single intravenous injection of 45 mg of crystalline sperm desoxyribonucleic acid into the mouse the spenic desoxyribonuclease activity was significantly increased. A similar enhancement of desoxyribonuclease activity was observed following the injection of a viable suspension of Candida guilliermondi. The results obtained following the injection of other microorganisms and the possible significance that altered spleen desoxyribonuclease activity has during systemic infections will be discussed.

1763. RELATIONSHIPS BETWEEN ROUTE OF ANTIGEN INJECTION AND HEMOLYSIN RESPONSE IN RABBITS. Laurence R. Draper and Dieter H. Süssdorf (intr. by W. H. Taliaferro). Dept. of Microbiology, Univ. of Chicago, Chicago, Ill.

The serum hemolysin response in intact or splenectomized rabbits varied with the route of injection of a single dose (approximately 1.6×10^9/kg rabbit) of heated sheep erythrocyte stromata. As compared to intact, intravenously injected rabbits, intact rabbits attained higher peak titers when injected into the femoral bone marrow or liver, had approximately equal titers when injected intramuscularly, intraperitoneally, intrasplenically, into the mesenteric lymph nodes or hind foot pads, and had lower titers when injected into the appendix, kidney fat or subcutaneous tissues of the lumbar region. In these groups, the timing and rate of

hemolysin production were similar. The response following splenectomy compared to that of intact rabbits was similar after injection into the peritoneal cavity, muscle, kidney fat, liver, appendix or subcutaneous tissues of the lumbar region, was lowered but not delayed after injection into the mesenteric lymph nodes or hind foot pads, and was both lowered and delayed after injecting i.v. or into the bone marrow. These results indicate: 1) that the appendix, kidney fat and subcutaneous tissues of the lumbar region do not contain important antibody-forming sites and that drainage from these areas does not concentrate antigen in efficient antibody-forming sites, 2) that nonsplenic sites located in or receiving antigen from the liver, muscle, peritoneum and hind foot pads function if enough antigen is concentrated therein and 3) that the response is dependent upon the presence of the spleen only after injecting i.v. or into the bone marrow.

1764. PERMEABILITY OF ISOLATED LYMPH NODE CELLS IN RELATION TO DELAYED TYPE SKIN SENSITIVITY. H. N. Eisen, M. Kern* and W. T. Newton.* Div. of Dermatology, Dept. of Internal Medicine, Washington Univ. School of Medicine and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.

Although there is strong evidence that induction of contact skin sensitization by simple chemical sensitizers is dependent on their combining chemically with tissue protein, conjugates prepared in vitro from a variety of proteins and sensitizers are extremely poor inducers of this type of sensitivity. This anomaly would be understandable if protein conjugates which form in vivo occupy sites which are not accessible to conjugated proteins prepared in vitro and then injected. As a preliminary examination of this hypothesis the permeability of isolated guinea pig lymph node cells to several dinitrobenzenes and to inulin has been studied. Expressing permeability as a ratio of water volume occupied by the penetrant in a packed cell pellet to the total pellet volume, the results were: 2,4-dinitrophenylbovine serum albumin (DNP-BSA), 0.25; inulin, 0.35; 2,4-dinitrobenzenesulfonate, 0.60; ϵ-N-DNP-lysine, 1.10; 2,4-dinitrochlorobenzene, about 2.50. With the latter substance C^{14}-labeled about 8% of the added material was not extracted with aqueous or organic solvents and probably was combined chemically with cell protein. The DNP-BSA space is the same as the theoretical value (0.25) for interstitial space between maximally packed spheres. It appears, therefore, that little, if any, DNP-BSA penetrated into cells; however, the simpler dinitrobenzene derivatives and inulin penetrated in varying degrees, or were concentrated by cells (values > 1.0). Normal cells and cells with the capacity to transfer skin sensitivity to dinitrobenzenes, gave the same permeability values for DNP-BSA, inulin, and ϵ-N-DNP-lysine, but the latter cells appeared to have a slightly greater space for dinitrobenzenesulfonate.

1765. LONG CHAIN FORMATION BY A STRAIN OF GROUP A STREPTOCOCCUS (TYPE 30) IN PRESENCE OF HOMOLOGOUS ANTISERUM. Richard Ekstedt and Gene H. Stollerman (intr. by Guy P. Youmans). Depts. of Bacteriology and Medicine, Northwestern Univ. Med. School, Chicago, Ill.

A strain of group A type 30 streptococcus was observed to grow in extremely long chains in liquid media (serum or Todd-Hewitt broth) to which homologous (type 30) rabbit antiserum was added. Addition to the media of rabbit antisera to heterologous types of streptococci failed to produce long chain growth. The long chain effect was destroyed by absorption of the antiserum with organisms of homologous type but was unaffected by absorption with organisms of heterologous types. The reaction disappeared at dilutions of antisera above 100-fold. Heating the media and antiserum at 56°C did not destroy the long chain effect. Addition of trypsin to the culture media prevented long chain formation. A survey of several strains of group A streptococci of the same and other types (6, 12 and 13) did not reveal the long chain effect. So far this phenomenon has been observed only with one strain of type 30. After 2-3 hr of growth the long chains of streptococci formed striking capsules. Although capsule formation was not dependent upon type specific antibody and was also observed in control cultures grown in normal serum, the morphology and size of the capsule were more striking in the long chains grown in homologous antiserum. Wright stains of the capsule revealed an amorphous or fibrillar eosinophilic structure containing deeply basophilic granules and septa of characteristic morphology.

1766. ANTIBODY RESPONSE OF RATS, MICE, AND GUINEA PIGS TO BOVINE SERUM ALBUMIN AS MEASURED BY ANTIGEN BINDING CAPACITY TEST. Richard Studley Farr. Dept. of Anatomy, Univ. of Pittsburgh, Pittsburgh, Pa., and Dept. of Medicine, Univ. of Chicago, Chicago, Ill.

The antibody response of rats, mice and guinea pigs to heterologous serum proteins is generally considered to be poor as measured by the precipitin reaction. The technique previously described (Fed. Proc. 15: 1955) for measuring the antigen binding capacity (ABC) of rabbit antibody directed towards bovine serum albumin (BSA) has been adapted to be used with rat, mouse and guinea pig antibodies. Since only 0.5 ml of antiserum is needed for the test, determinations can be made on the serum from individual mice. The ABC is an expression of the number of globulin sites which are capable of combining with BSA and is based on the primary antigen-antibody bond rather than the secondary aggregation of complexes as in the precipitin test. The ABC test depends upon the fact that BSA is soluble in 50% saturated ammonium sulfate whereas nonprecipitating BSA-antibody complexes formed in extreme antigen excess are insoluble under identical conditions. There is no indication that the ammonium sulfate fractionation procedure disturbs the antigen-antibody equilibrium to a significant extent. Preliminary experiments indicate that the antibody response of rats, mice and guinea pigs to BSA injected with Freund's adjuvant is considerable. The quality of the antibody produced in these 3 species as measured by the average dissociation constant obtained from the ABC test will also be discussed.

1767. ELUTION OF POLLEN ANTIGENS FROM TANNIC ACID TREATED ERYTHROCYTES. Richard J. Feinberg* and John A. Flick. Dept. of Microbiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.

Previous studies have shown that Boyden's technique of adsorbing protein antigens on tanned sheep erythrocytes can be used successfully with pollen antigens. It was observed that short ragweek (SR) treated cells, upon standing for prolonged periods of time, lost titer in the hemagglutination reaction with pollen antiserum. This loss of titer was notes with some antigenic fractions but not with others, and is probably due to slow elution of several antigenic components as indicated by the following observations. The supernate contained eluted antigen as seen by its ability to produce wheals in reactions in allergic persons. The addition of 1.15 μg of pollen extract protein nitrogen to each tube of a

series of 2-fold dilutions of SR antiserum decreased the hemagglutination titer from 2560 to <20. When the supernate was withdrawn from antigen-cells after standing 36 hr, the resuspended cells showed agglutination to full titer 2 hr after their addition to serial serum dilutions. If these same cells were incubated another 36 hr the system again showed a decrease in titer indicating further elution of free antigen. When serially diluted antibody and tanned antigen-cells were incubated separately for 36 hr and then combined, the decrease in titer was still noted. In all the above experiments the cells were washed sufficiently to free the supernate of detectable antigen prior to setting up the tests. It is believed that the avidity of short ragweed antigenic components for tanned RBC differ and also that tanned cells adsorb more antigen than is necessary to show hemagglutination.

1768. COMPARATIVE INHIBITION OF ANAPHYLAXIS IN MICE BY TRANQUILIZERS AND OTHER DRUGS. Charles L. Fox, Jr., Julia M. Einbinder* and Carl T. Nelson. Depts. of Surgery and Physiology, New York Med. College, Flower-Fifth Avenue Hosps. and Dept. of Dermatology, Columbia Univ. College of Physicians and Surgeons. New York City.

Various classes of drugs with specific pharmacologic properties were studied to determine their ability to prevent fatal anaphylaxis in sensitized mice. Drugs were investigated with adrenolytic and hypotensive properties: apresoline and Regitine; vasoconstrictor or hypertensive agents: epinephrine, norepinephrine and serotonin: sedatives: barbiturates; tranquilizers: reserpine, chlorpromazine, prochlorperazine, meprobamate; and anti-inflammatory drugs: salicylates, phenylbutazone and some newly available steroids with adrenocortical properties. The drugs were administered prior to challenge; the interval depending upon the rapidity and duration of their pharmacologic actions. Of this large group of compounds only certain of the steroids (Fed. Proc. 15: 55, 1955), 9 alpha-fluoro-hydrocortisone, and two tranquilizing agents with antiserotonin properties, reserpine and chlorpromazine, protected against fatal anaphylaxis. Protective doses of the latter compounds produced marked sedation. The union of antigen and antibody was not prevented inasmuch as protected animals were refractory to challenge for at least 1 wk. Rather, the shock syndrome following the immunologic reaction was blocked. (Aided by grants DA-49-007-MD-628, Dept. of the Army, and H-1650, Natl. Insts. of Health, PHS.)

1769. DURATION OF IMMUNITY TO EPIDEMIC TYPHUS INDUCED BY LIVING AVIRULENT R. PROWAZEKI, STRAIN E. John P. Fox, Martha E. Jordan* and Henry M. Gelfand.* Section of Epidemiology, Dept. of Trop. Med. and Public Health, Tulane Univ. School of Medicine, New Orleans, La.

As part of its evaluation as an immunizing agent to protect man against epidemic typhus, a living avirulent variant of R. prowazeki (strain E) was given to 125 volunteers in January 1951. At intervals since then available volunteers were bled for antibody studies and small groups were recruited which, with appropriate nonimmune volunteers as controls, were given intradermal challenge inocula consisting of living, unmodified R. prowazeki. Both clinical and serologic response to challenge were carefully observed. Aureomycin or, more recently, achromycin was used routinely after the 2nd day of fever to terminate such disease as developed. Previous publications have reported observations made through the 3rd year postvaccination. To be reported are the 4th and 5th year observations. All but 3 of 25 sera collected 4 yr after vaccination contained demonstrable neutralizing antibody although CF antibody had disappeared completely in most cases. All 3 exceptions were sera from the few men who, given the smallest doses of strain E, failed to develop detectable immune response immediately after vaccination. One of these participated in the 4th year challenge experiment and, together with both nonimmune controls, developed typical febrile illness after a challenge which was resisted completely clinically by 3 other vaccinated men. The 5th year observations are being initiated currently and the results will be reported in the paper as read.

1770. NUCLEAR LOCALIZATION OF A FACTOR FROM DISSEMINATED LUPUS SERUM. G. J. Friou, S. C. Finch, and K. D. Detre. (Intr. by John R. Paul). VA Hosp., West Haven, Conn. and Dept. of Internal Medicine, Yale Univ. School of Medicine, New Haven, Conn.

In vitro studies of localization of human serum in normal mouse tissue sections were done with a modified fluorescent antibody technique. Serum from all of a large number of patients with disseminated lupus erythematosus caused nuclear localization of fluorescent antihuman globulin. Many other serums were studied, including patients with related diseases, infections and drug reactions. Of this large group localization similar to that observed with lupus serum was encountered only with serum from 2 patients with unusually severe hypersensitive reactions to drugs.

1771. ANAPHYLACTIC SHOCK INDUCED BY SOLUBLE ANTIGEN-ANTIBODY COMPLEXES IN UNSENSITIZED NORMAL GUINEA PIGS. Frederick G. Germuth, Jr. and George E. McKinnon. Dept. of Pathology, Johns Hopkins Med. School, Baltimore, Md.

The allergic lesions of protracted anaphylaxis develop at a time when antibody forming in the tissues is undergoing rapid combination with excess antigen present in the surrounding fluid and circulating plasma. In previous studies, it has been suggested that antibody may be transported to the tissues in the form of soluble antigen-antibody complexes. The present study was undertaken to determine the role, if any, of soluble antigen-antibody complexes in the pathogenesis of allergy. The results show that supernatant fluids from antibody-excess antigen mixtures in vitro are capable of eliciting symptoms characteristic of anaphylactic shock in normal unsensitized guinea pigs. The evidence strongly indicates that the active material is a soluble antigen-antibody complex. 1) But for minor exceptions, only supernates from antigen-antibody mixtures prepared in antigen excess are capable of eliciting shock. 2) Shock can be produced by mixtures of purified antibody (gamma globulin) and excess antigen. 3) The further addition of excess antibody to supernates originally prepared in antigen excess removes their anaphylactogenic properties. 4) Soluble antigen-antibody complexes, prepared by the dissociation of washed immune precipitates by alkali in the presence of additional antigen, are capable of producing shock. 5) The amount of antibody contained in soluble antigen-antibody complexes capable of producing shock is similar to the amount required in passive anaphylaxis produced by the simultaneous injection of antigen and antibody.

1772. RESPONSE OF ESTABLISHED CELL STRAINS TO EASTERN EQUINE ENCEPHALOMYELITIS VIRUS IN RELATION TO CELL ACTIVITY.

G. O. Gey, F. B. Bang, M. Foard* and M. Svotelis.* Johns Hopkins Univ. School of Medicine and School of Hygiene and Public Health, Baltimore, Md.

Established rat and human cell strains have been demonstrated to vary in their susceptibility to this virus. Following inoculation of virus on roller tube cultures of these cells and incubation with continuous rolling during 6 hr, there was incomplete absorption even when a high cell/virus ratio obtained. Unabsorbed virus was detected by both embryo infectivity and plaque techniques. The variation in viral susceptibility of the different cell lines will be compared with established variation in cell activity as determined by growth rate, rates of particle (yolk) and fluid incorporation. Other factors involved in measurement of the absorption of virus in the cultures will be discussed.

1773. REPLICATION OF POLIOVIRUS IN PRIMATE CELLS MAINTAINED UNDER ANAEROBIC CONDITIONS. George E. Gifford* and Jerome T. Syverton. Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.

Evidence has accumulated in the literature to indicate that energy for virus synthesis in mammalian virus-host cell systems must be obtained from cellular aerobic processes. Contrariwise, we find that poliovirus replicates in strain HeLa human epithelial cancer cells and in normal monkey kidney cells maintained in vitro under strict anaerobic conditions. For example, when 40 TCIU of poliovirus were inoculated into cultures of 200,000 HeLa cells, after 50 hr of anaerobic incubation the titer was $10^{5.7}$ TCIU while after an equal time of aerobic incubation the titer was $10^{7.0}$ TCIU. This discrepancy in yield was found to be due to an increased latent period of virus development under anaerobic conditions as compared to aerobic conditions. The yield of poliovirus per infected cell was found to be the same under the two conditions. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis, Inc.)

1774. MECHANISM OF A PERSISTENT ADENOVIRUS INFECTION. Harold S. Ginsberg. Western Reserve Univ. School of Medicine. Cleveland, Ohio.

Establishment of an adenovirus infection of HeLa cells in tissue culture and persistence of the infection through multiple serial passages was previously reported (Fed. Proc. 15:589, 1956). To maintain this infection so that the cytopathogenic evidence of viral infection was "masked" a fluid which contained 40% human serum was employed. Although the evidence obtained suggested that the essential function of serum was to stimulate rapid cell growth, action of specific antibody or serum nonspecific inhibitor could not be discounted. The effect of antibody and animal serum virus inhibitor on viral multiplication and upon the cytopathogenic effect of adenoviruses was therefore investigated and the results form the basis of this report. After infection of HeLa cells with a large amount of type 4 adenovirus a high concentration of specific immune rabbit serum neither reduced viral multiplication nor cytopathogenic effect of virus. When 10 ID_{50} of virus was employed, however, immune serum markedly diminished the viral cytopathic effect but did not reduce viral propagation appreciably. A 10% concentration of normal rabbit serum, although unable to neutralize virus, did diminish the viral cytopathogenic changes. Ten percent normal horse serum, which contained a small amount of virus inhibitor, similarly lessened the characteristic pathologic injury by adenovirus. Rabbit, horse or human serum, 10% in maintenance mixture, markedly stimulated HeLa cell growth. These data indicate that certain sera, whether or not antibody or virus inhibitor was present, "masked" adenovirus cytopathogenic effects although viral multiplication was not reduced.

1775. VIRUS SUSCEPTIBILITY OF SERIALLY CULTIVATED HUMAN HEART TISSUE CULTURES. Anthony J. Girardi (intr. by M. R. Hilleman). Microbiological Research Fndn, Bethesda, Md.

Tissue cultures were prepared from a biopsy specimen of the right auricular appendage of adult human heart. This tissue culture line has been propagated serially for more than 1 yr. The cells in the cultures were of a fibrocytic type for the first 5 mo of cultivation and then progressively changed over a period of 5-6 wk to an epithelial-like form. The susceptibility of the culture line to a number of viruses will be reported.

1776. INTEGRATION OF HUMAN SERUM AND PLASMA FRACTION DIFFUSION PATTERNS IN AGAR COLUMNS. William G. Glenn and Anna G. Garner (intr. by Alan A. Boyden). School of Aviation Medicine, USAF, Randolph AFB, Tex.

Several characteristics for precipitin systems in agar columns are detailed for both simple and complex diffusion patterns. The reactants were pooled human serum, Cohn plasma fractions and antihuman rabbit sera. A Serum Agar Measuring Integrator (SAMI) is explained. This instrumentation enables semiautomatic quantitation of zone densities and diffusion distances to be recorded in graphic form. Consistent location of albumin and beta globulin systems in antihuman rabbit sera was confirmed by absorption and SAMIgraphic analyses. The greater sensitivity of this procedure compared to zone electrophoresis was demonstrated by fraction IV-7. Proposed criteria for establishing serological correspondence between diffusing proteins by zonal characteristics are presented.

1777. INHIBITION OF ADENOVIRUSES BY NORMAL ANIMAL SERA. Eli Gold* and Harold S. Ginsberg. Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Media for maintenance of cells in tissue culture are optimum when serum is included. During investigations to obtain a satisfactory fluid for propagation of virus in HeLa cells, it was observed that ox, calf or horse serum markedly inhibited the cytopathogenic effect of adenoviruses. Type 3 adenovirus, for example had a titer of $10^{-3.0}$ when titrated using a fluid containing 10% chick serum but only $10^{-1.25}$ when 10% ox serum was employed. Serum inhibitor not only reduced cytopathogenic effect of adenoviruses but also combined with and neutralized the agents. Types 4 and 7 adenoviruses were inhibited to the greatest extent; types 2 and 6 were least affected by the inhibitor. The inhibitor was present in highest concentration in ox serum. Experiments using ox serum helped distinguish inhibitor in that serum from other virus inhibitors in sera. The inhibitor studied was heat stable and formed a nondissociable complex with strains of types 1-7 adenoviruses. Inhibitor was not destroyed by a culture filtrate of Vibrio cholera (RDE), trypsin or incubation with infectious virus; nor was it adsorbed by kaolin or removed by dialysis. Recently isolated strains of adenoviruses were not inhibited to any greater extent than common laboratory strains. Addition of ammonium sulfate to half saturation resulted in precipitation of the inhibitory substance with the globulin fraction. Further studies are in progress for the characterization of the virus inhibitor. Although the nature of the

virus inhibitor is not yet known, these data indicate the importance of the composition of fluids employed for propagation of adenoviruses in tissue culture.

1778. SPREADING EFFECT OF HYALURONIDASE ON FREE TUMOR CELLS IN PERITONEAL FLUID (ASCITES TUMOR). Horace Goldie, Calvin A. Brown,* George L. Sampson* and Charles S. Thurston.* Lab. for Expl. Oncology, Nashville, Tenn.

In clinical practice the use of hyaluronidase is recommended for resorption of effusions in tissues and cavities but it is contraindicated for injections in cancerous areas. We have tested the above indication and caution in experimental animals. Groups of 25 mice received intraperitoneally on 3 successive days, starting 24 hr after tumor inoculation, doses of 25 (group A), 50 (group B), 75 (C), and 150 (D) TR units of Wydase (hyaluronidase): 25 (E) remained untreated (controls). On the 5th day peritoneal fluid was withdrawn from all mice and they were killed for autopsies. In groups B, C and D the amount of tumor cell implantation was considerably higher than in controls. In groups C and D the amount of ascitic fluid was reduced by 25 to 50%, but only in group D was the tumor cell concentration per cmm considerably lower (by 50 to 75%) than in controls. It is concluded that only very high doses of Wydase on 3 successive days induced partial absorption of ascitic fluid and promoted implantation of free tumor cells into serous surfaces of peritoneal cavity. After 3 injections of 1000-3000 U/g this invasion of tissues by free tumor cells from the fluid was apparently compensated by increased multiplication of remaining free cells, but repeated injections of 6000 U disrupted this balance to disadvantage of free cells. (Supported by grants from American Cancer Society, CH25B, and Natl. Cancer Inst., PHS, C2080).

1779. CYTOTOXIC EFFECT OF COMPLEMENT WITH RABBIT ANTISERA DIRECTED AGAINST HELA CELLS GROWN IN RABBIT SERA. Gerald Goldstein* and Quentin N. Myrvik. Dept. of Microbiology, School of Medicine, Univ. of Virginia, Charlottesville.

A line of HeLa cells adapted to growth in media containing rabbit sera as the sole nonsynthetic nutrient was employed in a study of its antigenic constituents. Aqueous extracts of HeLa cells in the 7th through the 15th passage in rabbit sera, and similarly prepared extracts of HeLa cells grown in media containing human sera, were used to immunize rabbits. Cultures of uniformly dispersed HeLa cells underwent characteristic cellular changes and subsequent growth arrest when incubated (15 min to 2 hr) with complement and cytopathogenic antibody (anti-HeLa cell rabbit serum). Complement is required for the lethal action of cytopathogenic antibody of HeLa cells, as cytopathogenic antibody alone produced reversible cellular changes without subsequent growth arrest. Sera from rabbits immunized with extracts of either line of HeLa cells produced cytopathogenic changes and growth arrest in either line of HeLa cells. In contrast, anti-human serum rabbit sera did not produce detectable cellular changes or inhibition of growth of HeLa cells. These observations indicate that the antigen (s) stimulating the production of cytopathogenic antibody is a constituent of HeLa cells and was not lost during adaptation to growth on media containing rabbit sera.

1780. ENHANCEMENT OF ANTIBODY PRODUCTION BY INJECTION OF TUBERCULIN IN SENSITIVE RABBITS. Robert A. Good, Richard M. Condie, Gail Thompson and Donna R. Jensen (intr. by Dennis W. Watson). Univ. of Minnesota, Minneapolis.

Studies by Johnson et al. (Fed. Proc. 13:499, 1954) and Condie et al. (Proc. Soc. Exper. Biol. & Med. 90: 355, 1955) showed that simultaneous injection of Gram-negative bacterial endotoxins and simple protein antigens result in enhanced antibody production to the latter. Observations by Stetson (J. Exper. Med. 101: 421, 1955) that reactions to Gram-negative bacterial endotoxin are similar to reactions of specifically sensitized rabbits to tuberculin suggested that injections of tuberculin in tuberculin-sensitive rabbits might enhance capacity to form circulating antibody. To test this hypothesis the following experiment was performed. Four groups were employed. Group I: tuberculin-sensitive rabbits injected with 200 mg of tuberculin + 20 mg bovine serum albumin i.v.; group II: tuberculin-sensitive rabbits injected with 20 mg BSA; group III: nonsensitive rabbits injected with 200 mg tuberculin + 20 mg BSA; group IV: nonsensitive animals injected with 20 mg BSA. Rabbits were bled 12 days following antigenic stimulation. The immune response was evaluated by comparison of amount of antibody produced by the rabbits in each group as measured by quantitative immunochemical technique. Rabbits of group I produced significantly more antibody against bovine serum albumin than did those of any other group. Data also suggested that tuberculin-sensitized rabbits and nonsensitized rabbits injected with tuberculin produced more antibody than did rabbits injected with BSA alone. These data are interpreted as indication that the reaction to tuberculin in tuberculin-sensitized rabbits was an adjuvant effect on production of precipitating antibody. (Aided by grants from the PHS, Helen Hay Whitney Fndn., American Heart Assoc. and Minnesota Heart Assoc.)

1781. CROSSREACTION OF TYPE II ANTIPNEUMOCOCCAL SERUM WITH DEXTRANS. Joel W. Goodman* and Elvin A. Kabat. Depts. of Microbiology and Neurology, Columbia Univ., Neurological Inst., New York City.

Since dextrans crossreact with type II antipneumococcal serum, an investigation of the crossreacting unit on the dextran was undertaken. The precipitin curves of three dextrans—(1) N236, 95% α 1,6 linkages and 4% α 1,3 like linkages; (2) B1355-S-4, 57% α 1,6 linkages, 34% α 1,3 like linkages, and 9% α 1,4 like linkages; (3) B1299-S-3, 50% α 1,6 linkages and 50% α 1,4 like linkages (periodate oxidation (Jeanes et al. J. Am. Chem. Soc. 76:5041, 1954)—were set up with type II antipneumococcal horse serum containing about 3.3 mg anti SII N/ml. At the maximum N236 precipitated about 550 μg antibody (Ab)N/ml, B1355-S-4 about 600 μg AbN/ml and B1299-S-3 about 500 μg AbN/ml, indicating that a similar structural unit was responsible for most of the crossreactivity. The capacity of various oligosaccharides to inhibit precipitation of about 26 μg N of the crossreacting type II antibody by 27 μg N236 was assayed by the quantitative precipitin technique. Amounts in micromoles giving 50% inhibition were: isomaltohexaose 0.165; isomaltopentaose 0.170; isomaltotetraose 0.210; α-methyl isomaltotrioside 0.250; isomaltotriose 0.275; α-methyl isomaltoside and panose 0.375; isomaltose 1.2; maltose 33; trehalose >45 (31% inhibition); gentiobiose >60 (22%); cellobiose >75 (21%); α-methylglucoside 12; β-methylglucoside >124 (13%); α-methylmannoside >100 (9%); glucose 42; galactose >110 (45%). These findings suggest that most of the dextran crossreactivity is produced by a sequence from the nonreducing end of at least two glucoses linked α 1,6 plus a second α linkage. The presence of 1,4 linkages appears to reduce rather than increase

inhibiting power. The small increases in inhibiting efficiency of the larger 1,6 linked oligosaccharides may be due to glucose units farther along the chain in homologous polysaccharide and suggests that the size of the combining region on the crossreacting fraction of type II antibody may be comparable to that found for human antidextran. (Aided by grants from the Natl. Science Fndn. and the William J. Matheson Commission.)

1782. SPECIFICITY OF REACTION OF CHICKEN ANTISERUM IN HIGH NACL CONCENTRATIONS. Morris Goodman and D. S. Ramsey.* Detroit Inst. of Cancer Research, Detroit, Mich.

Chicken antiserum in contrast to mammalian yields far more potent reactions in 8-13% NaCl than in 1% NaCl. In view of the reported coprecipitation of immune chicken alpha globulin with antibody, an investigation of the specificity of the high salt chicken antiserum system was undertaken. Antisers were produced in mature hens to human albumin, gamma globulin and orosomucoid. When an antiserum was absorbed in either 8 or 13% NaCl with an equivalence zone quantity of antigen, its electrophoretic pattern showed a marked decrease in the gamma area but otherwise was almost identical to the pattern afforded by the unabsorbed antiserum. There was no reduction of alpha globulin. The physicochemical properties of the chicken antibody molecules, themselves, would seem to account for the increased specific precipitation that occurs on increasing the NaCl concentration of the chicken antiserum reaction system. This same phenomenon of increasing precipitation was exhibited by the purified chicken gamma globulin which could be recovered from chicken antiserum by salting out at 32% NaCl and which contained almost all of an antiserum's precipitin activity. The supernatant, containing all the antiserum's alpha globulin, showed no coprecipitating activity. Antibody rich gamma globulin fractions were also isolated by differential salting out between 15 and 32% NaCl. The various gamma fractions were stable in 8 and 12% NaCl. The various gamma fractions were stable in 8 and 12% NaCl but some showed instability in 1% NaCl. These latter yielded in the antibody excess zone of certain antigenic systems coprecipitation of nonspecific gamma globulin in 1% NaCl but not in 8 or 12% NaCl.

1783. COMPARISON OF ADENOVIRUS GROWTH IN A VARIETY OF HUMAN TISSUE CULTURES INCLUDING THREE HELA CELL LINES. J. Thomas Grayston, Mabel E. Smith and Mary A. McCarthy (intr. by Clayton G. Loosli). Dept. of Medicine, Univ. of Chicago, Chicago, Ill.

Previously prepared and frozen stock types 3 and 4 adenovirus log dilutions to 10^{-8} were inoculated into test tube tissue cultures of several different human continuously propagated lines. The tubes were observed under the microscope for cytopathogenesis every 2 or 3 days for a total of 14 days' incubation. Previous isolation work had suggested that our HeLa cell line 1 (obtained from Dr. J. T. Syverton in 1953) was less susceptible to adenovirus than our HeLa cell line 2 (obtained from Microbiological Associates, Inc., Washington, D. C. in 1954). In a series of titrations line 2 showed cytopathogenesis with higher dilutions of both types 3 and 4 adenovirus. At 14 days, line 2 was positive at 10^{-5} with type 3 and 10^{-4} with type 4. Line 1 supported growth 1-2 logs less with both adenovirus types. The sensitivity of Puck's clone S3 HeLa cell line was found to resemble most closely the less susceptible line 1. When 2nd passages were made at 14 days the titer increased 100-fold within 6 additional days' incubation to as high as 10^{-7} in line 2. The following commercially available human cell lines showed susceptibility to adenovirus similar to the HeLa cell line 2: intesting (Henle), Detroit 6, KB (Eagle), conjunctiva (Chang) and J-111 (Osgood). The Chang liver cell was less susceptible. These results indicate that not all HeLa cell lines are equally satisfactory for isolation of adenovirus and that several human cell lines are as sensitive as HeLa to adenovirus.

1784. RELATIONSHIP BETWEEN INSUSCEPTIBILITY TO PHAGOCYTOSIS AND VIRULENCE OF CERTAIN KLEBSIELLA PNEUMONIAE STRAINS. Herbert E. Hall* and James C. Humphries. Dept. of Bacteriology, Univ. of Kentucky, Lexington.

The virulence of three M state, type A strains (69M, 108M, ScM) and one S state strain (108S) of Klebsiella pneumoniae was titrated in mice using the LD_{50} endpoint. The in vitro phagocytic susceptibilities of the strains were determined under various test conditions using the percentage of phagocytosis (%P) by mouse polymorphonuclear leucocytes as the criterion of susceptibility. The 4 strains differed markedly in virulence and in phagocytic susceptibility and there was a very high degree of correlation between relative virulence and insusceptibility to phagocytosis. Strain 69M was by far the most virulent (LD_{50}, 2.0×10^2) and was almost completely insusceptible to phagocytosis (%P, 4). Strain 108S was practically avirulent (LD_{50}, 4.1×10^6 and 5.6×10^7, respectively) and also showed proportionate intermediate degrees of phagocytic susceptibility (%P, 22 and 79, respectively). The relative phagocytic susceptibilities of the 4 strains were found to be unaffected by variations in the time of incubation or the ratio of bacteria to phagocytes. It would appear that the differences in virulence for the mouse of these 4 Klebsiella strains may be adequately explained on the basis of their demonstrated differences in susceptibility to phagocytosis by the polymorphonuclear leucocytes of this animal.

1785. IMMUNIZATION AND CHALLENGE OF HAMSTERS TO JAPANESE B VIRUS BY WEST NILE INFECTION PLUS JBE VACCINE. W. McD. Hammon and Imam Z. E. Imam. Dept. of Epidemiology and Microbiology, Grad. School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

Satisfactory immunization against Japanese B (JBE) virus is not presently available. It was our purpose to determine the effectiveness in experimental animals of a basic immunity to an important group B encephalitis virus by a primary infection with West Nile (WN) supplemented by an injection of an otherwise ineffective injection of JBE inactivated virus vaccine. Hamsters were immunized by a variety of procedures including WN infection, one or more injections of JBE vaccine and a combination of WN infection plus JBE vaccine. Following cortisone treatment to render animals highly susceptible to peripheral injection of JBE virus, the immunized and control hamsters were challenged subcutaneously with serial dilutions of virus. Serological responses were followed as well as mortality. Results with a combination of WN virus infection plus 1 or 2 injections of JBE vaccine encourage us to anticipate that if a strain of WN virus can be found that is sufficiently avirulent for man, subsequent injection with JBE vaccine might serve as a satisfactory immunizing procedure.

1786. DEVELOPMENT OF COMPLEMENT FIXING ANTIBODY IN ANIMALS RECEIVING LIVING AND INACTIVATED POLIOVIRUS. J. Donald Harr and Joel Warren. Div. of Biologics Standards,

Natl. Insts. of Health, Bethesda, Md.

Potency of formalinized poliomyelitis vaccine is currently based upon the neutralizing antibody response of vaccinated monkeys. This procedure has certain objections from the standpoint of economy and facility. In view of the relative speed and simplicity of the CF test and the demonstrated ability of poliomyelitis vaccinated guinea pigs and chicks to develop neutralizing antibodies, we have examined the sera of these species for CF antibody. Live monotypic antigens were prepared from infected monkey kidney tissue cultures and concentrated 30x in the ultracentrifuge to increase their reactivity. Postvaccination sera taken at increasing intervals was prepared in guinea pigs, monkeys and chicks. Guinea pig antisera to live virus were prepared for purposes of comparison. In several experiments log dilutions of vaccine were injected to establish dose extinction curves. Sera were tested by a conventional tube CF procedure with overnight fixation in the cold. There is little or no CF antibody in monkey sera 1 wk after the last of 3 weekly doses of vaccine at a time when neutralizing antibody is present, whereas a fairly consistent response occurs in guinea pigs 1 wk after the last of 2 doses injected 14 days apart. This response is proportional to dosage and related to virus type. Comparisons will be presented of the level of CF and neutralizing antibodies, of CF response as related to vaccine potency and of response to living and inactivated poliovirus.

1787. QUANTITATION OF ANAPHYLAXIS IN MICE. William E. Harris and John D. Fulton (intr. by L. P. Gebhardt). Dept. of Microbiology, School of Aviation Medicine, USAF, Randolph AFB, Tex.

It is common knowledge that hemoconcentration accompanies most types of shock. A marked rise in the hematocrit of anaphylactic mice has been observed. In order that the effect of various treatments on anaphylactic mice could be studied, a procedure for obtaining quantitative estimates of the reaction was developed. Female, Swiss-Webster mice, weighing 20-25 gm, were hypersensitized with 2 i.p. injections of alum precipitated bovine serum albumin plus Hemophilus pertussis vaccine. They were administered an intravenous challenge of bovine serum albumin plus H. pertussis vaccine 8 days later. To measure anaphylaxis, a modification of the standard microhematocrit procedure was utilized. Before challenge, the tail of the mouse was served and samples of the blood were collected in microprecipitin capillary tubes. Following challenge, hematocrits were taken at 5, 10 and 15 min. Hematocrit change was determined by subtracting the mean prechallenge value from the mean postchallenge value, and dividing by the mean prechallenge value. It was observed that the mean hematocrit rise in groups of 20-30 mice is a quantitative function of antigen concentration. The relationship appears to be of a sigmoidal nature. Statistical analysis of the ascending portion of the curve indicates that the degree of hematocrit rise is a linear function of the $\log_{10}$ dose antigen concentrations.

1788. SEROLOGICAL DIAGNOSIS OF ROCKY MOUNTAIN SPOTTED FEVER AND MURINE TYPHUS. David F. Hersey* and Charles C. Shepard. Virus and Rickettsia Section, Communicable Disease Ctr., Public Health Service, Montgomery, Ala.

Rocky Mountain spotted fever and murine typhus were studied as experimental infections in animals and natural infections in man. Block titrations of complement fixing antigen (method 1 of Topping and Shepard. Pub. Health Rep. 61:701, 1946) showed that the apparent antigen titer increased during the course of the disease, and that the usual unit of antigen (as it is ordinarily determined against a late convalescent serum) failed to detect antibody in early convalescent sera. Increasing the antigen concentration to 8 units made possible earlier detection of antibody. A commercial antigen could usually fix complement only with late sera. Thus it was observed that antibody changed in the course of the disease so that it fixed complement with lesser amounts of method 1 antigen and began to react with the commercial antigen. The importance of standardizing an antigen with an early rather than a late convalescent serum becomes apparent. A comparative study in human patients was carried out using the following serologic tests, which are arranged in order of their effectiveness in detecting antibody in these diseases: the complement fixation test (CF) using 8 U of method 1 antigen, Chang's ESS test, CF with 8 U of commercial antigen, CF with 2 U (the ordinary concentration) of method 1 antigen, Weil-Felix and CF with 2 U of commercial antigen.

1789. RESPIRATORY ILLNESS IN VOLUNTEERS GIVEN LIVE ADENOVIRUS INTRAMUSCULARLY. M. R. Hilleman, R. E. Hodges,* M. S. Warfield* and S. A. Anderson.* Dept. of Respiratory Diseases and Special Operations Branch, Walter Reed Army Inst. of Research, Washington, D. C.

In connection with investigations of living adenovirus vaccines, 6 human volunteers were inoculated intramuscularly with 1 ml of a pool comprising equal parts of types 3, 4 and 7 adenovirus propagated in Henle's strain of human embryonic intestine tissue culture. The cultures used to prepare adenovirus were maintained in serum-free mixture 199 during viral growth and the final material for human injection was exhaustively tested for sterility and safety. All subjects receiving the virus developed an acute respiratory illness which began 2 or 3 days postinoculation and lasted 4 or 5 days. The illness was characterized by fever, inflammation of the mucous membranes of the eyes, ears, nose and throat, hyperplasia of the submucosal tissues of the conjunctivae and pharynx, and hypertrophy of the cervical lymph nodes. The disease resembled the naturally occurring "ARD" or febrile catarrh with or without conjunctivitis as described for pharyngoconjunctival fever. Additionally, 3 of the 6 volunteers experienced a febrile toxic reaction which followed several hours after inoculation of virus. All volunteers except one, who had received killed vaccine previously, showed marked increase in neutralizing antibody against types 3, 4 and 7 virus and CF antibody as well. Virus recovery attempts were negative and were believed to be related to the very rapid antibody increase which appeared to be incompatible with excretion of viable virus.

1790. KINETICS OF FORMATION AND DISAPPEARANCE OF INTERMEDIATES IN HEMOLYSIS BY COMPLEMENT. Louis G. Hoffmann,* Manfred M. Mayer, Herbert J. Rapp* and Jose R. Vinas.* Dept. of Microbiology, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.

A kinetic method has been developed for measuring the formation and disappearance of the known intermediates, $EAC\overline{1}_{,4}$, $EAC\overline{1}_{,4,2}$ and E* throughout the course of the hemolytic reaction. By this technique the characteristics of action of complement (C') from any species can be ascertained from a single experiment and comparative evaluation can be made, provided that the components of the C' in question and those of guinea

pig C' can be substituted for one another. The method furnishes relative functional measurements of the components of C' and, in addition, yields information on their kinetic behavior. Results are far more informative than conventional component titrations which yield highly inaccurate values of dubious significance in a functional sense. The validity of such kinetic flow analyses has been ascertained on guinea pig C', and the results of application to the investigation of monkey and human C' will be shown. (Supported by a grant from Natl. Science Fndn. and by Contract ONR-248-17, Office of Naval Research.)

1791. SOURCES AND METHODS IN PREPARATION OF BLOOD GROUP SUBSTANCE A. L. M. Horger,* E. B. Gerheim and H. R. Maxwell.* Pharmacology Dept., Sherman Labs., Detroit, Mich.

Blood group specific substance A was prepared from porcine sources using modifications of methods suggested by Gerheim et al. (Proc. Soc. Exper. Biol. & Med. 72:394, 1949), Morgan and King (Biochem J. 37:640, 1943) and Goebel (J. Exper. Med. 68:221, 1938). The activity of the A-substance was determined with the isoagglutination test and the nitrogen and carbohydrate contents were determined by the micro-Kjeldahl and resorcinol methods, respectively. The materials used as sources of A-substance were Armour Stomach Substance Powder, Armour Pancreatin Powder, and Parke-Davis Pepsin (1:3000). The A-substances prepared from the stomach powder and from Pancreatin were comparable in activity and composition whereas that prepared from pepsin contained only about 1/20 of the activity. In addition, the pepsin product was found to contain a greater percentage of nitrogen and a smaller percentage of carbohydrate than the products prepared from the other sources. The method of preparation of the A-substance was modified by the use of solox, a phenol-ethanol mixture or ethanol as the fractionating agent and trichloracetic acid, the Folin-Wu method or the Sevag method for deproteinization. Fractionation with solox and deproteinization by the Folin-Wu method yielded a high potency product and has the added advantage of being a simple, inexpensive procedure. Preliminary attempts to further purify the A-substance by the use of ion exchange resins were unsuccessful.

1792. A NEW RUSSIAN VIRUS. Dorothy M. Horstmann and Elias E. Manuelidis.* Section of Preventive Medicine and Dept. of Pathology, Yale Univ., New Haven, Conn.

In 1950, a poliomyelitis epidemic occurred in Kazakhstan, USSR. From the pooled feces of 2 paralyzed children in this epidemic, Voroshilova and Chumakov isolated a virus which produced paralysis in monkeys, suckling mice and cotton rats, and was in addition weakly cytopathogenic in tissue cultures of human fibroblasts. No cross immunity could be demonstrated between the newly isolated virus and the 3 types of poliovirus. They proposed the strain be considered type IV poliovirus. Subsequent studies by Gard and Habel identified the mouse pathogenic material as Coxsackie A-7 virus. We have studied the behavior of this virus, kindly supplied to us by Voroshilova and Chumakov, in rhesus monkeys inoculated by various routes, and in 2 orally inoculated chimpanzees. The latter animals developed inapparent infections, as evidenced by rises in antibody, without virus being detected in their feces during the month after feeding. In monkeys, the virus produced only mild signs, with ataxia, tremors and weakness as characteristic features. With the aid of cortisone, a more severe disease was produced, and the virus has been carried through 7 serial monkey passages. The 6th passage monkey cord still produces paralysis in suckling mice. The pathological picture as studied in 13 monkeys is similar to that found in poliomyelitis, both as to type of neuronal lesion and distribution, but the Russian strain produces minor lesions fairly consistently in areas not involved in poliomyelitis, including the temporal and occipital lobes.

1793. ACTION OF INFLUENZA VIRUS ENZYMES ON HUMAN ERYTHROCYTE STROMA AND ON SOLUBLE INHIBITORS OF VIRAL HEMAGGLUTINATION. Calderon Howe. College of Physicians and Surgeons, Columbia Univ., New York City.

A lipopolysaccharide fraction containing virus hemagglutination receptors and blood group antigens was concentrated from human erythrocytes by ultracentrifugation. Its content of sialic acid, measured by the Bial and direct Ehrlich reactions, and its capacity to inhibit "indicator" virus hemagglutination, were compared with the same properties of beef submaxillary gland mucopolysaccharide (Heimer and Meyer, Proc. Nat. Acad. Sci. 42:728, 1956), human brain mucolipid (Rosenberg, Howe and Chargaff. Nature 177:234, 1956), and sputum mucoid (Rose, Erlanger and Howe. Unpublished). Influenza viruses (PR8, Lee, GLB, PR-301, and mouse brain-adapted WS) were concentrated from freshly harvested infective chorioallantoic fluid by ultracentrifugation. Liberation of dialyzable sialic acid from the soluble mucoids and from the stroma fraction by concentrated virus was correlated with loss of hemagglutination inhibitory capacity. Some degree of correlation was also found between the amount of sialic acid liberated and the hemagglutination inhibitory potency of the unaltered substrates. Neither blood group A antigen in stroma fractions from group A_1 donors nor blood group B antigen in sputum mucoid from a group B secretor were affected by exposure to concentrated virus. Culture filtrates of Clostridium tertium (Howe, MacLennan and Kabat. Fed. Proc. 15: 592, 1956) failed to liberate dialyzable sialic acid from the stroma fraction, or to affect its viral hemagglutination inhibitory capacity.

1794. PHYSICAL AND CHEMICAL PROPERTIES OF ANTICOMPLEMENT. W. S. Jeter and Albert P. McKee. Dept. of Bacteriology, Univ. of Iowa, Iowa City.

Sera containing antibody to complement were analyzed for activity as antibody protein and as carbon-14 from radioactive glycine labeled specimens. Increase in gamma globulin content was measured by Tiselius electrophoretic determinations on sera before and during the immunization process. Electrochromatographic fractionation of sera was accomplished. Results of these studies give further characterization of this antibody both in its activity and properties.

1795. TISSUE-LOCALIZING RESPONSE TO INJECTED ANTIGEN IN ACTIVE AND PASSIVE IMMUNITY. Reuben L. Kahn. Dept. of Dermatology, Univ. of Michigan, Ann Arbor.

Quantitative studies of the tissue-localizing response to injected protein antigen indicate that the ratio of the response of skin to muscle is approximately the same in normal and in passively immunized animals, but is considerably higher in actively immunized animals. The author's quantitative tissue-localizing method was employed. Horse serum was first used to immunize (or sensitize) rabbits and standardized horse serum antitoxin was then injected into a given tissue, to determine the capacity of that tissue to localize the

toxin-neutralizing (serum globulin) fraction and hence, serum proteins. Active immunization was accomplished by 2 injections of horse serum about 8 days apart, and passive immunization by intravenous injections of 20 cc of the immune rabbit serum/kg of body weight. The tissue-localizing response was determined by injecting a given number of units of diphtheria antitoxin into skin or muscle, simultaneously with a separate injection of 50 MLD of diphtheria toxin (Invariably lethal for rabbits), and finding the number of neutralizing units that will just protect the animal from toxin death. The greater the localizing response of the injected tissue, the more is the escape of the antitoxin prevented for the neutralization of the toxin, and the larger the number of units required for protection. When the antitoxin was injected into the skin, the requirements for protection were 15-20 units for normal rabbits, 1000-1500 U for the actively immunized and 75-100 U for the passively immunized animals; when injected into skeletal muscle, the requirements were 5-10 U for normal rabbits, 75-100 U for actively immunized and 25-50 U for passively immunized animals. This high ratio of the localizing response of skin to skeletal muscle noted only in active immunity, suggests the existence of an immunologic tissue capacity not transferable to passive immunity.

1796. A DISC METHOD FOR TITRATION AND NEUTRALIZATION OF POLIOVIRUS. Seymour S. Kalter. Virus and Rickettsia Section, Communicable Disease Ctr., Public Health Service, Montgomery, Ala.

A combination of the plaque method of Dulbecco (Proc. Nat. Acad. Sci. 38:747, 1952) and discs as used in antibiotic sensitivity tests offers a simple method for the study of certain cytopathic viruses in tissue culture. Monkey kidney cells were grown in screw-capped bottled (Hsiung and Melnick. Virology 1:533, 1955). The agar overlay as recommended by Youngner (J. Immunol. 76:288, 1956) was used in this experiment. Discs approximately 6 mm were cut from filter paper using an ordinary office paper punch and sterilized and dried in an autoclave. Several different manufactured filter papers were employed but no significant variations in results were noted. Discs containing antiserum were used either wet, i.e. immediately after dipping into the desired dilution of antiserum, or after lyophilization. Virus discs were used in the wet form as consistent results could not be obtained with lyophilized preparations of poliovirus. There is suggestive evidence, however, that other viruses may prove to be more stable when treated in this manner. For inoculation of the virus, a sterile disc held with forceps was moistened by touching it to the surface of the desired virus dilution and then placing it on the agar. To observe the neutralizing capacity of a serum, the disc containing the serum was placed on the agar surface and the disc impregnated with virus was then placed on top of the serum disc. Virus titrations and neutralizations made in this manner compared favorably with those made by the usual tube method. The reaction was specific and as yet no evidence of cross reactions has been observed among the polioviruses by this method.

1797. A STUDY OF HERPES SIMPLEX VIRUS BY THE PLAQUE TECHNIQUE IN RABBIT KIDNEY MONOLAYERS. Albert S. Kaplan (intr. by Melvin Cohn). Dept. of Bacteriology, Univ. of Illinois, Urbana.

Monolayer cultures, prepared in Petri dishes, were inoculated with herpes virus, overlaid with agar, and incubated at 37°. Plaques make their appearance by the 2nd day of incubation and reach their maximum number and size (1-2 mm) by the 4th-6th day. Approximately 50% of the virus is adsorbed in 90 min. One plaque is produced by a single infective particle, as shown by the linearity between virus concentration and number of plaques. Growth of virus in monolayers, with a virus:cell ratio of 10^{-2}, shows a latent period of 8 hr, after which free virus appears and rises exponentially. With a virus:cell ratio of 5, the latent period is 8 hr and the exponential virus increase lasts for the next 8 hr· the final yield at 28 hr was 280 plaque-forming particles (PFU) per cell. In experiments on the growth of virus in cell suspensions, with a virus:cell ratio of 1.6, there was a latent period of 7 hr, an exponential increase of virus for 5 hr and a yield of 100 PFU/cell. With a virus:cell ratio of 64, there was a latent period of 5 hr, an exponential increase for 5 hr and a yield of 126 PFU/cell. The reduction in the latent period at high virus:cell ratio suggests that more than one virus particle can participate in virus growth.

1798. IMMUNOFLUORESCENT REACTIONS OF HUMAN SERA WITH ISO-ANTIGENS IN HUMAN HEART TISSUE: AB BLOOD GROUP SUBSTANCES AND CARDIOLIPIN; EVIDENCE OF A THERMOLABILE ANTIBODY TO CARDIOLIPIN ASSOCIATED WITH INFLAMMATION. Melvin H. Kaplan. House of Good Samaritan, Children's Med. Ctr., and Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.

An immunofluorescent technique has been devised which permits the study of reactions between human sera and isoantigens in heart tissue. Blood group isoantigens A and B were localized in capillary walls, endothelium and adventitia of blood vessels, and in the connective tissue stroma. The specificity of the reaction was confirmed by its complete abolition after absorption of reactive sera with heart tissue homogenates or packed red cells of the homologous blood group. A second antigen detected in heart tissue was that reactive with Wassermann-positive sera. This staining reaction was localized mainly within the sarcoplasm of the myocardial cell, particularly between the myofibrils and adjacent to the cell membrane, with only traces of staining present in the connective tissue stroma and blood vessels. Absorption of such sera with beef cardiolipin (VDRL) resulted in considerable reduction of staining, whereas absorption with human heart tissue homogenates prevented staining completely. This sarcoplasmic pattern of staining was observed also with acute-phase sera from patients with rheumatic fever, rheumatoid arthritis and subacute bacterial endocarditis. In such sera, a thermolabile complement-fixing antibody for beef cardiolipin was found and could be quantitated after decomplementing the sera with an immune precipitate. The titer of this antibody showed striking correlation with the sedimentation rate and C-reactive protein and may reflect the presence of acute inflammation in these patients.

1799. ADJUVANT ACTION ON ANTIBODY PRODUCTION OF BACTERIAL ENDOTOXINS. Phyllis Kind, Yi-chi Mei, and Arthur G. Johnson (intr. by W. J. Nungester). Dept. of Bacteriology, Univ. of Michigan, Ann Arbor.

Studies on the means by which bacterial endotoxins augment the synthesis of antibody to protein antigens have been extended with experiments designed to define more precisely the time interval with respect to administration of antigen, during which endotoxin would exert its adjuvant effect. It has been shown previously

(J. Exper. Med. 103:225, 1956) that endotoxin administered with antigen results in a marked increase in antibody levels. In contrast, endotoxin given 4 days after antigen did not enhance antibody production. The results of current investigations show that endotoxin given to rabbits 5 hr before each of 3 injections, at 3-day intervals, of bovine serum albumin results in little if any, enhancement of antibody levels as determined by the quantitative precipitin technique. However, endotoxin administered 5 hr after injection of bovine serum albumin enhanced antibody levels by 5-fold over animals receiving bovine albumin alone. These data were confirmed and extended using the rate of antigen elimination as an index of antibody formation. Thus, endotoxin given 12 or 6 hr prior to radioiodinated human serum albumin failed to increase the rate of antigen elimination over animals receiving albumin alone. On the other hand, endotoxin administered either simultaneously, 24 or 48 hr after antigen, resulted in enhanced antibody production, as reflected in rapid removal of antigen from the circulation. The adjuvant effect of endotoxin on in vitro production of antibody by splenic cells is being investigated and the results will be discussed.

1800. ANTIGENIC SPECIFICITY OF PATHOLOGICAL MACROGLOBULINS. Leonhard Korngold and Gerda Van Leeuwen.* Sloan-Kettering Inst. for Cancer Research, New York City.

Macroglobulins from patients with macroglobulinema are antigenically related to the macroglobulin of normal serum gamma-1 globulin (Korngold. Fed. Proc. 15:1956). Recent studies with antinormal gamma-globulin sera have shown that the macroglobulins have several antigenic groupings in common with the 7S gamma-globulin, and that macroglobulins from different macroglobulinemia patients differ among each other and from normal macroglobulin. When these proteins are compared by the gel diffusion technique (Korngold. J. Immunol. 77:1956), spurs and double spurs are formed; all pathological macroglobulins are deficient in antigenic groupings with respect to normal macroglobulin. Hyperimmunization of rabbits with 3 highly purified macroglobulins produced potent antisera. After absorption with gamma-2 and beta globulins two lines were obtained with the homologous antigen; the curvature of the lines showed that both were produced by macroglobulins. The antisera cross-react with macroglobulins from other patients and normal macroglobulin (1 line). After exhaustive absorption with gamma-1 and beta globulins these antisera react only with the homologous (2 lines). These data indicate that the macroglobulins from patients with macroglobulinemia are structurally abnormal because: 1) they lack several normal antigenic groupings and 2) they possess groupings that are patient specific.

1801. DECAY OF DIFFERENT ANTIBODIES IN THE GUINEA PIG. Marian Elliott Koshland. Brookhaven Natl. Lab., Upton, N. Y.

From previous studies of antibody formation in lymph tissue and antibody accumulation in the blood, it was predicted that the half-life of circulating diphtheria antitoxin in guinea pigs was approximately 6 days. The decay of antitoxin has not been determined by both immunological and radioactive measurements in guinea pigs of different ages and after exposure to X-irradiation. An average half-life of 5.9 ± 0.2 days was obtained which was independent of the experimental conditions tested. In a similar kinetic analysis of cholera antibody synthesis, a half-life of approximately 3 days was calculated for circulating antibody. When the fate of I^{131}-labeled cholera antibody was determined experimentally, a value of 5.6 ± 0.5 days was obtained. This discrepancy between the derived and experimental results disappeared when the kinetic calculations were based on the total antibody response and not, as previously, on the difference between the total antibody and the naturally occurring antibody to the vibrios. Thus, these studies on cholera antibody decay indicate that there is continual synthesis of antibodies which react with the cholera antigen and that only anamnestic formation takes place on inoculation of the vibrios.

1802. VIRAL HEPATITIS: EXPERIMENTAL TRANSMISSION AND ATTEMPTS AT PREVENTION. Saul Krugman,* Robert Ward, Joan P. Giles* and Milton A. Jacobs.* Dept. of Pediatrics, New York Univ. College of Medicine, and Willowbrook State School, N. Y.

Since 1953 over 350 cases of hepatitis with jaundice were observed at an institution housing over 400 mental defectives. Epidemiological evidence suggested that the disease was probably infectious hepatitis. Studies were designed to determine: 1) if gamma globulin would prevent its spread and 2) if passive-active immunity could be induced. Results have shown a) that the protective effect of gamma globulin (0.01 ml/lb) was not dramatic and may have been significant only in the period from 3-7 wk after injection. This study is being repeated with an increased dosage of gamma globulin (0.06 ml/lb). b) No evidence of passive-active immunity has been observed. An attempt is being made to induce experimental passive-active immunity as follows: subjects have received gamma globulin followed by ingestion of active hepatitis virus. They will be tested for immunity later with active virus. The source of virus was a suspension of stool from jaundiced patients in the institution. This material was rendered bacteria-free by antimicrobial agents and heating at 56°C for 30 min. Inoculation of tissue cultures, baby mice and monkeys yielded negative results. Titration by mouth produced mild hepatitis with jaundice in 18 subjects following long incubation periods ranging from 39 to 67 days. The 50% infectivity end-point was about 1 gm of stool.

1803. PRODUCTION OF MULTIPLE ANTIBODIES IN HUMAN SUBJECTS FOLLOWING HYPERIMMUNIZATION WITH DIPHTHERIA TOXOID. William J. Kuhns. Central Blood Bank and Dept. of Pathology, Univ. of Pittsburgh, Pa.

Schick negative persons respond to one booster dose of diphtheria toxoid by forming high titers of precipitating and/or nonprecipitating antitoxin. Some of these persons may also form antibodies against trace amounts of nontoxic agents contained in the immunizing materials. In the present experiments multiple antibodies were demonstrated in 11 of 59 immune sera studied. Antitoxin titers were determined by means of the rabbit neutralization test. Precipitation tests in a semisolid medium, in the presence of both crude and purified toxoid, furnished information concerning the presence of more than one immune system. Additional related knowledge was provided in some instances by the behavior of the quantitative precipitation reaction. Human passive transfer studies were carried out on sera containing nonprecipitating antitoxin or antibody. Differentiation between passively transferred skin-sensitizing antitoxin or antibody was based upon the ability of only antitoxin to produce a local state of Schick negativity in the skin of Schick positive persons. It could then neutralize the delayed reaction of equivalent Schick toxin injected later. Absorption of 4 immu

sera containing precipitating antitoxin and precipitating impurity antibodies, using crude toxin-poor antigens followed by immunochemical analyses, as described above, made it possible to demonstrate relative amounts of antitoxin and impurity antibody N. In a serum containing nonprecipitating, skin-sensitizing antibodies and precipitating antitoxin, identification of individual components was possible following partial separation by electrophoresis. Comparisons between responses in immunized subjects who formed "single" and multiple antibodies of different specifications showed that the mean antitoxin titer was much higher in the latter group although the discrepancy in titers could not be accounted for by known differences related to past history or immunization procedures.

1804. ANTIBODIES FOR COXSACKIE VIRUSES IN MONTANA. David B. Lackman and J. Frederick Bell.* Rocky Mountain Lab., Natl. Insts. of Health, Hamilton, Mont.

Coxsackie virus antigens for complement fixation were prepared from moribund suckling mice according to the protamine-precipitation method of Melnick. Antisera used in the standardization of antigens were obtained from adult mice immunized with complement-fixing antigens. Sera collected during a poliovaccine study were tested to determine the experience of persons in Montana with Coxsackie viruses. In contrast to findings in neutralization tests, no increase in frequency of complement-fixing antibodies with age was observed. This indicates that complement-fixing antibodies probably represent a response to recent infection while neutralizing antibodies have a tendency to persist, thus giving rise to their accumulation with age. Forty-six percent of one series of 255 serums contained complement-fixing antibodies for Coxsackie viruses; 20% against group B, 9.8% against group A, and 16.1% against both groups A and B. It has not been possible to relate the presence of complement-fixing antibodies to any recognizable clinical syndrome. During this study the following types were found to contain a common antigen: group A, types 4 and 9, and group B, types 3 and 5. Antisera for these 4 types reacted to similar complement-fixing titers against 8 isolates obtained from a family slightly ill with an undifferentiated respiratory infection.

1805. SEROLOGICAL MUTANTS AND STRUCTURE OF PHAGE T5. Frank Lanni and Yvonne Thery Lanni.* Dept. of Bacteriology, Emory Univ., Ga.

T5 stocks contain mutants B^+F^-, which infect Escherichia coli strain B but not FCb, and F^+B^- with the converse properties (Bact. proc. 51, 1956). The wild-type (WT) infects both hosts with equal efficiency. Extensive cross-neutralization occurs in rabbit antisera against any of the viruses; however, quantitative differences are apparent among the viruses and among the antisera. 1) All sera neutralize F^+B^- approximately twice as fast as WT (assays on FCb). Hence, these 2 viruses probably differ in some organizational feature on the relevant antigen(s) rather than in qualitative antigenic composition. 2) WT antisera neutralize B^+F^- slightly more slowly than WT (assays on B), while B^+F^- antisera neutralize this virus 3-5 times as fast as WT. Hence, these viruses probably possess qualitatively different antigens and/or different proportions of multiple antigens. Cross-absorption experiments are in progress. 3) With WT, the estimate of serum-survivors in neutralization by any of the antisera depends on the assay host. The ratio "plaques on B/plaques on FCb" becomes greater than units at the outset of neutralization and thenceforth increases progressively. Analysis has shown that many or all of the initial virus particles pass through a stage in which they are effectively incapable of adsorbing to FCb but still capable of adsorbing to and infecting B. In conclusion, the serum-sensitive portion, probably the tail, of the T5 particle appears to contain an ensemble of somewhat independently functioning, not necessarily heterogeneous antigens, whose collective structure can vary mutationally in at least 2 distinct ways.

1806. EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE HOST CELL (HeLa)/POLIOVIRUS INTERACTION. Edna Larson,* William F. McLimans, Geoffrey W. Rake and C. V. Harding.* Wistar Inst. of Anatomy and Biology, and Univ. of Pennsylvania, Philadelphia.

An investigation has been made of the effect of environmental temperature on the host cell/virus interaction in a tissue culture system. Strain HeLa (Gey) experimentally infected with the MEF-1 poliovirus (type II) was incubated at 37°C for 1-1/2 hr after infection, after which time the cells were removed to 25°C. It has observed that the reduction of environmental temperature did not appreciably alter the ratio of replication of the virus in its intracellular phases, but did inhibit, nearly completely, the cellular release mechanism to the extent that very little extracellular virus could be detected during the first 24-hr period. Microscopic examination of coverslip preparations revealed typical cytopathology in most of the cells 24 hr after infection. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis.)

1807. MODIFICATION OF THE ANTIBODY RESPONSE BY INJECTION OF SPLEEN HOMOGENATES IN X-IRRADIATED RATS. Mariano F. LaVia,* Melba J. Robson* and Robert W. Wissler. Argonne Cancer Research Hosp. and Dept. of Pathology, Univ. of Chicago, Ill.

Exposure to 500 r of total body x-radiation will inhibit the response of rats to a single intravenous injection of a particulate antigen administered shortly after irradiation. In an effort to gain a better understanding of the mechanism by which recovery from this nonresponsive phase is accomplished, a series of experiments was carried out. Male albino rats were exposed to 500 r, total body x-irradiation. Immediately thereafter or within the next 3 days they received an intravenous injection of spleen homogenate containing 2×10^8 cells. Homogenates were prepared from spleens of "normal" rats and from spleens of rats which had been stimulated earlier with an intravenous dose of S. typhi vaccine known to produce pyroninophilic cellular proliferation. All animals received one intravenous injection of sheep erythrocytes at varying intervals after the spleen homogenate. Homogenates from both "normal" and "stimulated" spleens were capable of restoring partially the antibody forming capacity of irradiated rats, the protection afforded by "normal" spleen being somewhat greater. The peak titer of serum antibody was delayed in the irradiated spleen-injected rats. The histologic response in the spleen of the irradiated treated rats was studied in relation to the humoral antibody response. The role of spleen cells in protecting the antibody forming mechanism from acute irradiation damage will be discussed.

1808. PRECIPITATION REACTIONS IN AGAR WITH POLIOVIRUS CONCENTRATES. George L. Le Bouvier (intr. by John R. Paul). Section of Preventive Medicine, Yale Univ. School of Medicine, New Haven, Conn.

Type-specific precipitates can be formed in agar by

antibodies present in monkey antisera and antigens present in crude or purified concentrates of poliovirus grown in cultures of monkey kidney. More than one type-specific antigen-antibody precipitate may be produced. The character of the precipitate may be affected by treatment of the virus preparation with agents such as heat or Merthiolate. Type-specific precipitates have been obtained using human sera. Increases in circulating precipitin against the infecting type of poliovirus can be demonstrated in some poliomyelitis patients during the acute stage of the disease.

1809. ALTERATION OF ANTIGENICITY OF OVALBUMIN BY γ-RAYS. Charles A. Leone. Argonne Natl. Lab. and Univ. of Kansas, Lawrence.

Two times recrystallized ovalbumin, in the powdered state and 1% solutions, at 0°C, were irradiated in a high energy field of γ-rays (1.5-3.5 x 10^6 rep/hr). The solutions received dosages between 10^5 and 8.5 x 10^6 rep. The powdered proteins received dosages between 32 and 300 x 10^6 rep. At γ-ray dosages below 6 x 10^5 rep the capacity of irradiated ovalbumin to precipitate antisera against native is essentially unchanged from that of native, despite considerable physicochemical evidence of induced structural modification. At higher dosages there is a progressive loss of serological activity without a significant loss in protein, soluble at pH 7. Guinea pigs were inoculated, i.p., with a range of sensitizing amounts (0.001-30 μg) of irradiated or native ovalbumin and were challenged 28 days later with a 1 mg i.v. injection of native proteins. Irradiated, powdered ovalbumin progressively lost its capacity to sensitize. Some irradiated solutions had an improved capacity to sensitize guinea pigs to native proteins. The maximum potentiations, which varied between 30 and 100 times greater than the native, were obtained in solutions absorbing 6 x 10^5 rep. Larger doses of irradiation to solutions caused a loss in antigenicity. The potentiating component of the irradiated solutions is stable at the isoelectric point of ovalbumin, but is heat labile at 50°C for 1-1/2 hr. The synergistic effect was not obtained in intravenously sensitized guinea pigs.

1810. ACTIVATION OF A PROESTERASE ASSOCIATED WITH PARTIALLY PURIFIED FIRST COMPONENT OF HUMAN COMPLEMENT. I. H. Lepow, O. D. Ratnoff* and L. R. Levy.* Inst. of Pathology and Dept. of Medicine, School of Medicine, Western Reserve Univ., Cleveland, Ohio.

Previous investigations have indicated that the first component of complement (C'1) exists in human serum as a proesterase which may be activated either by antigen-antibody aggregates or, in the partially purified state, by adjustment of physicochemical conditions (Proc. Soc. Exper. Biol. & Med. 92:32, 111, 1956). The kinetics of the spontaneous activation of the partially purified proesterase (C'1) has been studied further by measuring the rates of disappearance of hemolytically-active C'1 and of appearance of an esterase hydrolyzing N-acetyl-L-tyrosine ethyl ester. Activation appears to be an autocatalytic reaction occurring optimally at pH 7.3-7.7. The rate of activation increases with increasing temperature in the range studied (0°-37°C); the energy of activation calculated between 10° and 25°C is 31,000 calories per mol. Activation is inhibited by 10^{-4} M ethylene diamine tetraacetate but not by ion-exchanging with amberlite IRC-50 (Na cycle). Activation is also inhibited by ionic strengths above 0.15, and by heat-labile a and heat-stable factors in human serum. The possible relation of these factors to components of complement will be discussed. The correlation obtained between C'1 disappearance and esterase appearance under the conditions investigated provides further direct evidence for the proesterase role of C'1.

1811. METABOLIC EFFECTS OF ANIMAL VIRUSES IN TISSUE CULTURE. Hilton B. Levy, Samuel Baron and Wallace P. Rowe (intr. by Karl Habel). Natl. Insts. of Health, Bethesda, Md.

In an attempt to study animal viruses in a manner comparable to bacteriophage, beginnings have been made in the use of animal viruses in tissue culture under conditions approximating a synchronous infection. A high ratio of inoculum virus/cell gives reasonable assurance that most of the susceptible cells will be infected at about the same time. One can then measure metabolic effects that are not confused by the simultaneous termination of infection and death in some cells and initiation of infection in others. With several viruses (poliovirus and adenovirus are reported here), an early increase in lactic acid production has been found. With type 3 poliovirus, for example, increased glycolysis occurs within an hour after infection, and sometimes earlier. The effects of virus infection on the uptake of radioactive glycine, and of radioactive inorganic phosphate is also noted early in infection. The qualitative nature of these latter effects, however, is different for different viruses. The relative effects of virus infection on the uptake of P^{32} and glycine-2-C^{14} into the acid-soluble, lipid, protein and nucleic acid components will be discussed.

1812. VARIATION IN VIRULENCE OF MOUSE-ADAPTED TYPE III POLIOVIRUS. C. P. Li and W. D. McBride. Div. of Biologics Standards and Natl. Inst. of Dental Research, PHS, Bethesda, Md.

In a previous paper now in press we have shown that mouse-adapted type III poliovirus could be made to lose or gain its mouse virulence (intraspinal inoculation) by varying the cell type used for cultivation. Mouse-virulent virus grown in monkey testicular tissue culture lost virulence after 1 or 2 passages and remained nonvirulent after 15 consecutive passages in the same tissue culture. However, the same mouse-adapted virus retained its mouse virulence after 6 passages through monkey kidney tissue culture. In this experiment the nonvirulent variant was transferred into 2 sets of monkey kidney tissue cultures: In set A, 199 medium was used as the maintenance medium. In set B, medium "E", consisting of 1 part of 5% bovine plasma hydrolysate and 3 parts of Hanks-Simms solution, was used. The pH of both media was adjusted to 7.4-7.5. At harvesting time, the pH of both media varied from 7.1 to 7.3. After more than 10 passages, repeated experiments showed the virus from set A had regained mouse virulence: i.e. inoculation of 10^5 $TCID_{50}$ paralyzed 7 of 10 mice; 10^3 $TCID_{50}$, 3 of 10 mice. Virus from set B remained nonvirulent: i.e. 10^6 $TCID_{50}$ paralyzed none of 10 mice. These results demonstrate that the medium for cell maintenance is also an important factor affecting the virulence of virus.

1813. ANTIBODIES FOR PATHOGENIC AND NON-PATHOGENIC SEROTYPES OF ESCHERICHIA COLI IN HUMAN COLOSTRUM AND SERUM. Robert B. Lindberg and Donald H. Hunter.* Dept. of Bacteriology, Walter Reed Army Inst. of Research, Washington, D. C.

In outbreaks of diarrhea due to pathogenic Escherichia coli in newborn infants it has often been noted that breast-fed infants succumb far less often to infection than do formula-fed babies. The implication that ingested antibody in colostrum might play a role in this

difference in susceptibility prompted a study of colostrum and serum specimens from 50 mothers. Antibody for 3 major pathogenic serotypes, 0-111, 0-55, and 0-127, was measured, together with that for 2 nonpathogenic serotypes, 0-17 and 0-18. The latter were selected because of their broad spectrum of cross-reactions for E. coli serotypes as demonstrated in hyperimmune sera. Antibody was measured using a hemagglutination system with an alkaline extract of bacterial cells as sensitizing antigen. Agglutinins for at least 1 serotype of E. coli were demonstrated in every specimen of serum and of colostrum, with maximum titers of 1:4096 in colostrum and of 1:128 in serum. Specificity of the reaction was indicated by showing complete removal of antibody in both colostrum and serum when these were absorbed with appropriate heated bacterial cells. The mean titers in colostrum were much higher than those in serum, and a much higher proportion of colostrum specimens reacted with pathogenic E. coli than was the case with the nonpathogenic serotypes. Titers of colostrum for pathogenic serotypes were several fold greater than the titers for the nonpathogenic serotypes. The potential significance of these findings is discussed.

1814. SEROLOGICAL STUDY OF PRIMARY ATYPICAL PNEUMONIA. Ch'ien Liu and James T. Heyl.* Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass., and Phillips Exeter Academy, Exeter, N. H.

In previous communications, the optimum conditions for the cultivation of primary atypical pneumonia virus (PAPV) in the chick embryo were described. These were based on the finding that serum from patients convalescent from PAP would react specifically with infected embryo cells as demonstrated by fluorescein-labeled antihuman γ-globulin (Liu and Eaton. Bact. Proc. 1955, p. 61; Liu, Eaton and Heyl. Bull. New York Acad. Med. 32:170, 1956). The virus multiplies in the bronchial epithelium, and isolation and cultivation are therefore favored by intra-amniotic infection of older embryos (13 days). A study of more than 75 patients from several outbreaks of primary atypical pneumonia during 1954-1956 was shown that the antibody responsible for the "staining" reaction appears during the 2nd or 3rd week and persists for more than 1 yr. It is readily distinguishable from cold agglutinins and from streptococcus MG agglutinins. Eighty percent of the cases, clinically diagnosed as PAP, showed rises in the titer of "Staining" antibody, indicating that this reaction is a reliable test for retrospective diagnosis. Eight strains of virus were isolated during the investigation. All cross-reacted indistinguishably with each other and with the Mac strain isolated by Eaton, Meiklejohn and Van Herrick in California in 1944. There were no reactions between embryos infected with PAPV and human or rabbit sera containing antibodies against influenza A, influenza B, Q fever, psittacosis, pneumonia virus of mice (PVM) or adenovirus types I-VI. Factors in human and guinea pig serum indistinguishable from complement greatly enhanced the staining reaction with human serum.

1815. SEROTHERAPY OF POLIOMYELITIS IN RHESUS MONKEYS. Oscar C. Liu, Joseph E. Carter and Bettylee Hampil (intr. by Kingsley M. Stevens). Dept. of Virology, Merck Sharp and Dohme Research Labs., West Point, Pa.

Serotherapy for poliomyelitis has been deemed impractical since the report of Bahlke and Perkins (J.A.M.A., 129:1146, 1946) who used gamma globulin probably of low antibody level. The availability of hyperimmune serum prepared by present immunizing agents has suggested the desirability of further exploration of the subject in the laboratory. In each experiment, 8-10 rhesus monkeys were used; 10-40 $TCID_{50}$ of Mahoney virus (equivalent to 3-13 MPD) were injected into the lumbar enlargement of the spinal cord. Half the animals were treated with antiserum intravenously (20 ml/kg), and the remainder, receiving no therapy, served as controls. Treatment was given at one of the following times after infection: 0, 24 and 48 hr. The antiserum obtained from rabbits immunized with multiple intravenous injections of Salk vaccine had a neutralizing titer of 1:50,000 vs 100 $TCID_{50}$ Mahoney virus. No monkeys died in any of the treated groups whereas the mortalities in the control groups varied between 75 and 100%. The incidence of paralysis in the treated was 60-100%; that of the controls 75-100%. However, the degree of paralysis was much milder in the treated animals and the disabled muscles were limited to the lower extremities even when therapy was delayed 48 hr. The controls became completely paralyzed between the 4th and the 8th day. Protection was achieved also by administering the antiserum either intraperitoneally (20 ml/kg) or intrathecally (0.8 ml/kg).

1816. EFFECT OF X-IRRADIATION ON THE ANTIGEN-HOST GENETIC RELATIONSHIP. Takashi Makinodan and Isabel Shekarchi.* Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

An attempt is being undertaken to test the validity of the importance of the antigen-host genetic relationship. It is felt that such studies would contribute to the apparent role of the recognition factor of the antibody-producing cell; that is, in what manner can an antibody-producing cell recognize that a substance is foreign and to what degree of foreignness? Although normal mice respond to rat antigen equally as well as sheep antigen, during the recovery phase following total-body X-irradiation the antibody response to the genetically more distant sheep antigen is greater than to the more closely related rat antigen. This was demonstrated when the agglutinin response was determined 1, 15 and 30 days posttreatment. The results further showed that pretreatment with 2-mercaptoethylguanidine HBr (MEG) of lethally X-irradiated mice is comparable to posttreatment with isologous bone marrow. Previous results have shown that injection of bone marrow into X-irradiated mice permits transplantation of RBC, granulocytes and platelet precursors, while difinitive evidence to show transplantation of antibody-producing cells is lacking. Hence, it can be deduced that MEG protects predominantly non-antibody-producing cells. Studies on bone marrow transplantation and the induction period of sheep and rat agglutinin production immediately following irradiation suggest existence of either types of antibody-producing cells or an antibody-producing cell with ability to recognize (a) homologous, (b) closely related heterologous and (c) distantly related heterologous antigens. In terms of these hypothetical properties, it is suggestive that the degree of irradiation destruction would be in the above order.

1817. EXTENT OF RESISTANCE INDUCED BY IMMUNIZATION AGAINST HISTOPLASMA CAPSULATUM. Stanley Marcus and Gilbert A. Hill.* Dept. of Bacteriology, College of Medicine, Univ. of Utah, Salt Lake City.

There is little information available concerning the quantitative extent of resistance induced by immunization or by chronic infection with H. capsulatum. The effect of immunization with organisms of varying

virulence and the effect of sublethal infection upon subsequent challenge has been studied. It was found that formalin killed vaccines prepared from 2 strains of H. capsulatum, distinguishably different in mouse virulence, increased resistance of the immunized animals to intravenous challenge with the more virulent strain of organism. The degree of resistance induced by the 2 strains was not significantly different. The extent of resistance induced, as determined by potency ratio calculation, was such that the $LD_{50/21}$ days for immunized animals was 5.3 times (95% confidence limits 1.2-23.0) that for controls. In other experiments, mice were sublethally infected with the less virulent, and subsequently challenged with the more virulent strain of H. capsulatum. Evidence indicating 100% infection of the sublethally infected group was derived from spleen cultures of 10% of this group killed prior to challenge. The extent of resistance induced by sublethal infection, as determined by potency ratio calculation, was such that the $LD_{50/21}$ days for infected animals was 7.7 times (95% confidence limits 2.8-21.3) that for controls. The experimental design allows direct comparison of the data. Under the conditions employed, immunization with killed vaccine and chronic infection both resulted in increased resistance; the extent of increased resistance induced by the 2 procedures was not significantly different. (Aided by a grant from the Office of Naval Research.)

1818. I^{131} TRACE IODINATION OF DIPHTHERIA TOXIN. S. P. Masouredis. Dept. of Pathology, and Central Blood Bank, Univ. of Pittsburgh Med. School, Pittsburgh, Pa.

The effect of 2 methods of iodination on biological activity, flocculation, precipitation and storage stability of diphtheria toxin has been studied. Method A involved use of free iodine obtained with nitrite (J. Immunol. 67:243, 1951) and method B involved use of I_3^- in phosphate buffer, pH 7.5 (J. Clin. Invest. 23: 357, 1944). Two preparations were obtained using method A. One preparation (A-1) contained 1.1 moles I per mole toxin, 0.76 μc I^{131}/μg toxin N and 346 μg N/ml. These values for the second preparation (A-2) were, respectively, 4.4, 6.92 and 419. Preparation B contained 1.2 moles I/mole toxin, 1.02 μc I^{131} μg N and 334 μg N/ml. The ability of all the I^{131} toxins to flocculate was quantitatively unimpaired except for qualitative differences in preparation A-2. All preparations contained from 0.36 to 0.44 μg N/Lf. The I^{131} toxin obtained by method A (A-2) at equivalence in the precipitin reaction brought down only 80% of the nitrogen brought down by the uniodinated toxin. The I^{131} toxin obtained by method B reacted identically to the uniodinated toxin in the precipitin reaction. In the range of antibody excess 84% of the total I^{131} was precipitated. Guinea pig toxicity of the toxin labeled with method A was markedly impaired. It required 3 (A-1) to 10 (A-2) times as much of the I^{131} toxin to kill a guinea pig as compared to the uniodinated toxin. The I^{131} toxin obtained by method B showed no loss of toxicity. Following 50 days of storage at 4°C, preparation A-2 lost 75% of its toxicity whereas uniodinated toxin and I^{131} toxin obtained by method B retained full toxicity following storage. (Supported by grants from the Public Health Service.)

1819. ACETYLATED RABBIT SERUM ALBUMIN, AN ANTIGEN IN RABBITS. Paul H. Maurer and J. Sri Ram.* Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pa.

N-acetylation of proteins using acetic anhydride in cold sodium acetate medium is generally believed to be a mild reaction, causing little or no change in the protein, other than the conversion of free amino groups into N-acetyl groups. We have previously observed that acetylated bovine serum albumin was different from native BSA in several physiochemical properties and appeared to be partially denatured. It was also found that although the free amino groups of the antigen appeared to be unnecessary for the BSA-anti BSA interaction, acetylated BSA exhibited a cross reaction of less than 50% with anti BSA, indicating that significant changes must have taken place in the protein during the acetylation reaction. In line with these findings we now have observed that acetylation of rabbit serum albumin has caused sufficient changes in RSA to make it antigenic in rabbits. While adjuvant immunization was found to be more effective, one course of immunization with alum precipitated antigen caused detectable antibody formation. Passive anaphylaxis tests in guinea pigs were 100% positive. Acetylated derivatives of BSA, HSA and RGG cross reacted with the rabbit antiserum obtained against acetylated RSA, while the unmodified proteins exhibited no such cross reaction. Cross reactions were also exhibited by the acetylated derivatives of RSA, HSA and RGG, with the rabbit anti acetylated BSA, indicating that part of the specificity of these antibodies was directed against sites on the protein containing the acetyl groups. These sites are apparently somewhat similar in various proteins.

1820. ANTIBODY FORMATION INITIATED IN VITRO. John M. McKenna* and Kingsley M. Stevens. Dept. of Virology, Merck Sharp and Dohme Research Labs, West Point, Pa.

Rabbits were given 10 μg of the lipopolysaccharide endotoxin of S. typhosa intravenously. This material, supplied by Dr. A. G. Johnson of the University of Michigan, enhances the primary response to protein antigens (Johnson et al. J. Exper. Med. 103:225, 1956). Twenty-four hour later, the spleens were removed, diced, and incubated for 1 hr at 37°C with 500 μg/ml of bovine-γ-globulin (BGG). After washing 5 times with Hanks' solution, the tissues were planted as previously described (Fed. Proc. 15:616, 1956); J. Immunol. In press). Antibody was titrated by a modification of the Boyden hemagglutination technique. After incubation for 24 hr, fluid titers averaged 26 and the agglutination could be specifically inhibited with BGG. Control titers averaged 3. This difference was highly significant by Student's t test ($P < 0.001$). From combining tests with antigen, it was estimated that a titer of 26 represented approximately 0.1 μg of antibody N. By use of the technique of Steiner and Anker (Proc. Nat. Acad. Sci. 42:580, 1956), modified for use as a completely in vitro system, titers up to 256 were secured in extracts of spleen fragments.

1821. MECHANISM OF ACTION OF EMULSIFIED VACCINES. Robert W. McKinney* and Fred M. Davenport. Epidemiology Dept., School of Public Health, Univ. of Michigan, Ann Arbor.

The adjuvant effect of mineral oil emulsions on antibody response can be conveniently studied in mice by comparing levels of antibody achieved after vaccination with aqueous and emulsified influenza virus vaccines. In this model system, it has been shown that the peak level of antibody reached 6 wk after vaccination is dependent upon depositing the vaccine in situ for about 16 days. The rate of breakdown of emulsified vaccines was investigated by determining the amount of antibody produced when the site of inoculation was removed or whole body irradiation given at intervals after vaccination. In addition, the rate of release of pa from water-in-mineral oil emulsions was determined.

Taken together, the results obtained indicate that release of antigen occurs as early as 8 hr after vaccination, and by 16 days the major portion of antigen responsible for determining the peak level of antibody has been liberated. A slow prolonged release of antigen from the site of inoculation seems critically important for attaining high antibody levels and for maintaining them over long periods of time, since removal at intervals after vaccination of the granulomas formed about the antigen depot is followed by decline in antibody levels. Data to support this interpretation were derived by experiments in which small amounts of aqueous vaccine were given repeatedly. The results obtained when aqueous vaccines were introduced into cotton pellets previously implanted subcutaneously emphasize the role of inflammation in enhancement of antibody production found with adjuvant vaccines.

1822. SUBMERGED CULTURE OF MAMMALIAN CELLS - THE SPINNER CULTURE. William F. McLimans, Eldon V. Davis,* Fletcher Glover,* Adrian Rake* and Geoffrey W. Rake. Wistar Inst. of Anatomy and Biology, and the Univ. of Pennsylvania, Philadelphia.

The design and application of a system (spinner culture) permitting the active proliferation of mammalian cells, as individual units, in the submerged state has been described. "L" cell, strain HeLa, and human conjunctival cells have been successfully cultivated in volumes of 25-300 ml. An "L" cell culture has been maintained in a state of continuous growth in the spinner system for over 3 mo. The cells proliferate as isolated units with little if any clumping. At no time has a proteinaceous precipitate been observed. It is felt that a more quantitative system is thus provided for determining the effects of viral proliferation, drug action, etc., on mammalian cells. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis.)

1823. INFLUENCE OF AMBOCEPTOR CONCENTRATION ON UPTAKE OF COMPLEMENT AT 0°C. David L. McVickar (intr. by A. F. Rasmussen, Jr.). Dept. of Infectious Diseases, School of Medicine, Univ. of California, Los Angeles.

It has been previously shown that erythrocytes sensitized at supraoptimal concentrations of amboceptor will lyse in the presence of C' at 0°, whereas erythrocytes sensitized at optimal amboceptor concentration undergo little or no lysis under similar conditions. Tests for residual amboceptor in the supernatants of suspensions of erythrocytes sensitized at varying concentrations of amboceptor indicate that even when 1000 hemolytic units of amboceptor are employed, greater than 99% of the amboceptor is adsorbed. The previously demonstrated linear relationship between maximal velocity of hemolysis of sensitized erythrocytes and the concentration of amboceptor reflects an increase in velocity of uptake of C' with increasing concentration of amboceptor. Thus, at amboceptor concentrations 50x optimal, 90% of the available C' (1000 U/ml reactivation mixture) is adsorbed by the time the erythrocytes are completely lysed (90 min), 50% of the total amount being taken up before hemolysis becomes detectable; whereas at optimal amboceptor concentration, less than 20% of the same amount of C' is adsorbed within 240 min, at which time only 8% hemolysis has occurred. The difference in rate and amount of uptake of the 4 components of C' by erythrocytes sensitized at different amboceptor concentrations will be discussed.

1824. RETICULOENDOTHELIAL EFFECTS OF SOME ATARACTIC AGENTS. R. M. Meier,* D. A. Boroff and J. H. Heller. Div. of Physiology and Microbiology, New England Inst. for Med. Research, Ridgefield, Conn.

Patients on some ataractic drugs have presented clinical evidence of increased incidence of infections and some refractoriness to antibiotic therapy. This pattern points to possible involvement of the reticuloendothelial system (RES). In order to investigate the role that drugs such as chlorpromazine, Pacatal (N-Methylpiperidyl-(13)-methylphenothiazine), reserpine and 2-methyl, 2-n-propyl-1,3 propanediol dicarbamate might play on the RES, studies were undertaken to investigate their effect on phagocytosis and resistance to calibrated infection. Studies were made of the phagocytic efficacy of the RES in mice treated with various drugs using the rate of disappearance of intravascular colloidal carbon to demonstrate the degree of depression of RES function. Certain drugs, of which chlorpromazine is the best example and which result in a higher incidence of infection clinically, depressed the phagocytic ability of the RES maximally, while another phenothiazine, Pacatal, did not show this effect. The ability to withstand a calibrated infection in mice is also reduced upon the administration of thos compounds which depress the phagocytic velocity of the RES. The changes in the RES due to these drugs appear to be parallel to the above mentioned observed clinical side effects and appear to be independent of such factors as hypothermia.

1825. INCORPORATION OF DEUTERIUM INTO DPNH-X. Josephine O. Meinhart and Marvin C. Hines (intr. by Charles A. Evans). Dept. of Biochemistry, Univ. of Washington, Seattle.

Triose phosphate dehydrogenase catalyzes the conversion of DPNH to a derivative designated as DPNH-X (Chaykin, Meinhart and Krebs. J. Biol. Chem. 220:811, 1956). When the reaction is carried out in D_2O, one deuterium atom is incorporated into DPNH-X. ADP-ribose obtained from this DPNH-X by hydrolysis does not contain deuterium. When the DPNH-X is converted to DPN and hydrolyzed with DPNase, all the deuterium is found in the nicotinamide. These results are consistent with the proposed structure of DPNH-X as a monohydroxytrihydronicotinamide analog of DPN. This structure is also supported by the previously reported finding of only one easily hydrogenated double bond in the pyridine moiety of DPNH-X, and the recently reported absorption spectrum of N-methyltetrahydronicotinamide (Marti, Viscontini and Karrer. Helvet. Chim. Acta 39:1451, 1956). Other evidence relating to the structure will be reported.

1826. EFFECT OF BRUCELLA ENDOTOXIN ON ADRENOCORTICAL FUNCTION IN THE DOG. J. C. Melby,* R. H. Egdahl* and W. W. Spink. Depts. of Medicine and Surgery, Univ. of Minnesota Med. School, Minneapolis.

Direct assessment of adrenocortical function in anesthetized dogs by the measurement of total minute output of free 17,21-dihydroxy-20-ketosteroids in adrenal venous blood was accomplished by adrenal vein cannulation. Prior to the administration of endotoxin, samples of adrenal venous blood for control studies were obtained in the resting animal and also following exogenous ACTH stimulation. Two hours after ACTH the endotoxin of Brucella melitensis (5 mg/kg of body wt) was given i.v. Venous adrenal blood was collected after 5 min and at hourly intervals thereafter for 3 hr. The animal in every experiment

demonstrated the picture of shock induced by Gram-negative bacterial endotoxin (Weil et al. J. Clin. Invest. 35:1191, 1956). ACTH was rapidly given i.v. again and adrenal venous blood was collected intermittently for several minutes. Intact dogs responded to ACTH stimulation before endotoxin as well as to the endotoxin with an immediate abrupt rise in the minute output of adrenal 17,21-dihydroxy-20-keto-steroids. The secretion of 17-hydroxycorticosteroids was elevated for 2 hr after endotoxin and feel thereafter toward normal levels. ACTH given after this produced an increase in the total 17-hydroxycorticosteroid output, but the response was significantly reduced compared to control responses. Hypophysectomized dogs failed to respond with an increased secretion of 17-hydroxycorticosteroids after endotoxin similar to the control dogs following endotoxin. In the hypophysectomized dog, the corticoid response to stimulation with ACTH was greater before endotoxin had been given than was the response to ACTH after administered endotoxin. Stimulation with ACTH increased corticoid output to a lesser degree as compared with the control response after endotoxin in the hypophysectomized dog. Apparently adrenal secretory response to exogenous ACTH is impaired in shock induced by endotoxin.

1827. ANTIBODY PRODUCTION IN TISSUE CULTURE. Maria C. Michaelides (intr. by A. H. Coons). Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.

Rabbits which had received a single subcutaneous injection of 160 Lf of fluid diphtheria toxoid in the footpad were given a 2nd similar dose about 4 mo later, when their serum titer was less than 0.005 μ/ml. These animals were killed at intervals after this 2nd injection, the homolateral popliteal lymph node was excised and fragments cultured in roller tubes (approximately 12 fragments in each tube, about 2 mm^3 each). The medium was Eagle's medium (J. Exper. Med. 102:37, 1955) in Hanks solution containing 25% normal rabbit serum, with antibiotics. The medium in the roller tubes was replaced at intervals of 3-4 days. The fluid withdrawn was tested for diphtheria antitoxin, using Boyden's technique of hemagglutination as modified by Stavitsky (J. Immunol. 72:360, 1954). Under these conditions continued antitoxin formation by the lymph node fragments was observed for as long as 26 days. In succeeding experiments, the time-interval between the 2nd injection of antigen and the removal of the lymph node was successfully shortened from 3 days to 2-1/2 hr. Since this short interval indicated that the conditions in the cultures were favorable for the development of the cells involved, the 2nd injection into the rabbit was eliminated and instead the 2nd dose of antigen was added directly to the lymph node fragments. In these preliminary attempts to stimulate a secondary response in vitro, a few of the tubes showed antitoxin formation on the 9th day. The maximum so far is equivalent to the formation of approximately 0.1 unit of antitoxin in a 3-day period.

1828. AN ANTIGENIC VARIANT OF TYPE 61 PNEUMOCOCCUS. Stanley E. Mills* and Henry P. Treffers. Dept. of Microbiology, Yale Univ., New Haven, Conn.

Laros (J. Infect. Dis. 85:263, 1949) reported that type 61 pneumococcus (P61) serially subcultured in cross-reacting type 31 antiserum yielded a stable, modified strain (M61). M61 showed no cross-reactivity for type 31, still cross-reacted with P61, and gained cross-reactivity for type 13, an activity not present in P61. During the subculture it was found that M61 could be first detected in from the 5th to the 9th transfer tube, and estimates of the total P61 population size attained averaged about $4(10)^9$ cells. M61 cells were scored in type 13 antiserum-agar pour plates. A precipitin ring formed around M61 but not around P61 colonies. In an attempt to study this population shift by a fluctuation analysis, P61 was grown in normal rabbit serum broth, agglutinated with type 31 antiserum, and the supernatants screened for M61 cells. No M61 were found by this technique from populations of P61 as high as $3(10)^{11}$ cells. It seemed that the appearance of M61 cells was not independent of the presence of antiserum. Purified preparations of P61 and M61 polysaccharides (SSSP61 and SSSM61) were obtained. Quantitative precipitin analyses with rabbit antisera to P61 and M61 revealed that SSSM61 absorbed 73% of the antibody to P61, while SSSP61 removed 61% of the M61 antibody. Preliminary monomer analysis by acid hydrolysis and descending chromatography revealed that both SSSP61 and SSSM61 yielded 4 identically moving spots; an amino sugar, a spot moving identically with mannose, and 2 as yet unidentified constituents.

1829. BIOLOGIC DISPOSITION OF DIHYDROCHOLESTEROL (DHC) IN THE RABBIT. E. H. Mosbach,* E. Halpern,* D. Harris* and F. E. Kendall. Columbia Univ. Research Service, Goldwater Memorial Hosp., and Dept. of Medicine, Columbia Univ., New York City.

Biologic disposition of dietary DHC and DHC-4-C^{14} was studied in rabbits. This sterol was absorbed less completely than cholesterol from the alimentary canal, but after 4-8 wk on a diet containing 1% DHC, serum levels up to 150 mg% DHC were produced. At that time, DHC accounted for over 60% of both the free and esterified sterol of the serum and liver, and for about 50% of the sterol content of the erythrocytes. The cholesterol content of the liver increased, but at a slower rate than the DHC concentration. Tracer studies showed that this accumulation of cholesterol was not due to a conversion of DHC into cholesterol. The total sterol content of muscle was not greatly changed, but DHC replaced as much as 50% of the cholesterol in this tissue. Rabbits fed DHC developed biliary calculi made up largely of glycine conjugates of cholanic acids. At the end of a 4-wk balance study with DHC-4-C^{14} the specific radioactivities of the liver sterols and of the bile acids (isolated from the biliary calculi) were identical, indicating that DHC can be converted into bile acids or closely related products. Radioactivity was also present in the fecal bile acids isolated during the course of the balance study, but DHC administration did not increase the excretion of bile acids over the amount excreted on a DHC free diet.

1830. EFFECT OF FORMALIN ON HUMAN RED CELL ANTIGENS. Merwin Moskowitz and Saul Carb (intr. by F. Joseph Murray). Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.

Human red cells were treated with 20% formalin in a phosphate buffer at pH 7 for 24 hr at room temperature. After such treatment red cells of type A, B, and Rh, and red cells rendered panagglutinable by periodate, were no longer agglutinated by their respective antisera. Such treated red cells, however, were able to absorb their specific antibodies to the same extent as normal red cells. Formalin acts on the red cell surface in a manner such that agglutination is inhibited but it apparently has no effect on the red cell antigens that have been studied. Implications of these findings will be discussed.

1831. EFFECT OF H. PERTUSSIS ON SENSITIVITY OF MICE TO SEROTONIN. J. Munoz and M. A. Greenwald.* Merck Inst. for Therapeutic Research, Rahway, N. J.

It is recognized that histamine plays an important role in anaphylactic reactions. Recently serotonin has been implicated in these reactions. In mice it has been shown that injection of H. pertussis produces an increase in sensitivity to histamine and a marked increase in their ability to become anaphylactically sensitive. In spite of these observations, the role that histamine plays in mouse anaphylaxis has not been established. It has been found, for example, that certain strains of mice that do not become sensitive to histamine after pertussis injection become sensitive to anaphylaxis. This and other observations point to another mechanism besides histamine action as the cause of anaphylaxis in mice. In an effort to uncover other substances that might be involved, it was decided to investigate the effect of pertussis on the sensitivity of mice to serotonin. It was found that after injection of pertussis cells mice become more sensitive to serotonin and that, contrary to what has been observed with respect to histamine, the sensitization seems to be independent of the strain of mice used. The increased susceptibility can be demonstrated 24 hr after injection of pertussis and reaches its peak by the 3rd day. The sensitivity persists for at least 10 days. The substance responsible for the production of serotonin sensitivity seems to be peculiar to pertussis, since other bacteria have failed to produce this sensitivity.

1832. ANTIGEN-ANTIBODY REACTIONS IN AGAR: IV. EFFECT OF ANTIBODY CONTENT AND OTHER FACTORS ON THE R' VALUE. J. C. Neff* and E. L. Becker. Div. of Immunology, Walter Reed Army Inst. of Research, Washington, D. C.

Using the Oudin serum agar technique, and employing a series of antigen concentrations over a wide range of antibody concentrations, the maximum concentration of antigen giving zero movement of the hand was determined and the ratio of this concentration to the antibody concentration, the R' value, was calculated. With the egg albumin-rabbit anti egg albumin and human serum albumin-horse anti human serum albumin systems, it was found that in high antibody concentration the R' value approximated the ratio of antigen to antibody in the precipitate at the antigen excess end of the equivalence zone, and conversely, at low antibody concentrations, the R' value approximated the ratio of antigen to antibody at the antibody excess end of the equivalence zone. The precipitate ratios were found by the usual quantitative precipitin technique. Curves given by various antisera to the same antigen were not always superimposable when the R' values were plotted against the antibody nitrogen values. Relationship of these findings to the determination of antibody concentration by measurement of band penetration will be discussed.

1833. ENHANCED ANTIBODY RESPONSE IN MICE IMMUNIZED WITH BOVINE SERUM ALBUMIN (BSA) INCORPORATED INTO PARTICLES MADE FROM HEMOGLOBIN. Eric L. Nelson (intr. by C. P. Miller). Dept. of Medicine, Univ. of Chicago, Chicago, Ill.

When lysates of washed erythrocytes from which all particulate material has been removed are mixed with appropriate concentrations of deoxyribonucleic acid (DNA) in dilute solutions (1/400 lysate, .25 mg/ml DNA at pH 5.0) and incubated at 37°C, spherical particles 1-10μ in diameter develop. Most of the hemoglobin and DNA in the mixture become concentrated in the particles. If a protein, polysaccharide is added to the hemoglobin-DNA mixture prior to incubation, 20-50% of the added component becomes incorporated into the particles. Particles containing BSA have been prepared by this procedure and employed as immunizing antigens. A single injection of BSA (0.3 mg/mouse) in the protein incorporating particles (PIP) yielded antibodies detectable to 1:5 dilution. Two s.c. injections 20 days apart resulted in anti-BSA titers greater than 1-100. Three injections of 1 mg BSA in solution did not elicit any detectable antibody response in mice. Effect of multiple injections of BSA-PIP and the route of administration on antibody response was determined. The s.c. route appears essential for a primary response in mice. Secondary responses occur as well with i.p. or i.v. injections. Antibody titers were measured by the ammonium sulfate technique of Farr, using the pooled sera from 10 mice for each group studied. Avidity constants were determined for each serum pool.

1834. DURATION OF HEMAGGLUTININ RESPONSE OF PATIENTS WITH SHIGELLOSIS AND SALMONELLOSIS. Erwin Neter and Donal Dunphy.* Depts. of Pediatrics and Bacteriology, Children's Hosp., and Univ. of Buffalo, Buffalo, N. Y.

Studies from this laboratory have revealed that the enterobacterial hemagglutination test is considerably more sensitive than the conventional bacterial agglutination method for the demonstration of the antibody response of children with infections due to salmonellae, shigellae, and enteropathogenic Escherichia coli. The present study was carried out to determine the duration of the hemagglutinin response of children with salmonellosis and/or shigellosis. It was found that within a period of 4 mo to 2 yr the hemagglutinin titers, markedly elevated during the acute infection, returned to normal levels in 16 out of 18 patients. Two of these children had attacks of both salmonellosis and shigellosis. In one of the remaining individuals the Salmonella choleraesuis hemagglutinin titer decreased substantially but did not return to normal levels, and in the other patient with Shigella flexneri dysertery the titers remained unchanged. Only the latter patient harbored the pathogen at the end of the observation period. No parallel changes in the titers of heterologous hemagglutinins were observed. The hemagglutinin titers were from 2- to 64-fold higher than those of agglutinins. It is concluded that, in the absence of the carrier state and of chronic or recurrent infection, the specific hemagglutinin response of the majority of children with salmonellosis and/or shigellosis (18 out of 20 infections) is maintained at high level for a period of less than 2 yr. The significance of the data for studies of enterobacterial infections by means of epidemiologic serology is discussed. (Aided by a research grant from the Natl. Inst. of Allergy and Infectious Diseases, PHS.)

1835. COMPLEMENT FIXATION STUDIES WITH AGENTS FROM PATIENTS WITH BOSTON EXANTHEM DISEASE. Franklin A. Neva and Mary F. Malone (intr. by T. H. Weller). Dept. of Tropical Public Health, Harvard School of Public Health, Boston, Mass.

Complement fixation was demonstrated utilizing sera and agents derived from patients with an exanthem disease that occurred in Boston (1951) and Pittsburgh (1954). A modified micro-method of Fulton and Dumbell using 2 drops of antigen and other reagents in

12 x 75-mm tubes was employed. Antigens produced in vitro were tested with acute and convalescent phase sera from patients and from immunized monkeys. Undiluted culture fluids gave irregular fixation of complement with specific serum; the concentration procedure of Svedmyr and co-workers (Proc. Soc. 79:296, 1952) was therefore used. A 40- to 90-fold concentration of infected fluids ($10^{-3.5}$ to $10^{-4.5}ID_{50}$) from cultures of human skin-muscle or lung yielded antigens that could be diluted up to 1:10 for optimum activity. No antigenic differences were found between agents isolated from Boston patients and those from Pittsburgh. A 4- to 16-fold rise in antibody was demonstrable in paired sera from patients of both cities convalescent phase titers varied between 32 and 256. Paired sera from 20 patients with other exanthematous diseases exhibited no change in C-F titers. The incidence of antibody in different age groups is under study. In diagnostic studies on the exanthem diseases the C-F test has proven to have advantages over the in vitro neutralization technique. (Supported by grants from the Public Health Service (E-1023-C) and from the Lederle Labs.)

1836. MECHANISM OF PASSIVE CUTANEOUS ANAPHYLAXIS. Abraham G. Osler, Mary M. Hawrisiak* and Zoltan Ovary.* Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.

This investigation is concerned with the detection and identification of immunological factors other than antigen and antibody which may mediate passive cutaneous anaphylaxis (PCA) in the albino rat. Correlative studies indicate a marked parallelism between the level of serum complement (C') and PCA. Thus, rats rendered deficient in serum C' by means of an in vivo, unrelated immune reaction lose their responsiveness to PCA. The restoration of the cutaneous reactivity and the return of serum C' follow a similar time course. PCA in untreated animals may be enhanced by the injection of fresh or ammonia-treated guinea pig or rat serum. Serum lacking hemolytic activity following treatment with zymosan is ineffectual. Na EDTA but not Na CaEDTA abolishes the cutaneous response. Data will be presented to show that PCA, as an immunological reaction, can be studied as a system of three variables, namely, antigen, antibody and a host factor which may be complement or one of its components.

1837. HUMAN BLOCKING-TYPE, SKIN-SENSITIZING ANTIBODY. N. C. Palczuk* and J. A. Flick. Dept. of Microbiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.

Sera were obtained from 3 persons immunized with a mixture of pneumococcal polysaccharides, types 2, 6 and 7. Two of the donors were atopic individuals; one was normal. These sera were tested by the Prausnitz-Küstner technique in a person sensitive to the polysaccharides. Serum sites upon challenge demonstrated an inhibition of wheeling and erythema as compared to the positive control sites. This suggested the presence of blocking-type antibodies. While undiluted sera gave this blocking effect, the same sera diluted 1:100 did not. This blocking property was observed in bleedings obtained over a 4-6 month period. After injection of skin sites the blocking phenomenon was demonstrable at intervals of 24, 48, and 72 hr, indicating fixation to the skin similar to that demonstrated in studies with reagins. All 3 immunized donors showed positive wheal and erythema reactions by direct skin tests to varying degrees with these polysaccharides. Serum treated with beta-propiolactone or by Seitz filtration, as well as serum carefully separated from the clot and untreated, produced the same blocking effect. In comparing serum heated for 4 hr at 56°C and unheated serum, there does not appear to be a loss or gain in blocking activity. Additional studies are being done to characterize this blocking-type antibody further and to determine whether it is manufactured by the body as such, or if it is another type antibody which is possibly modified in some way by the processing of the serum.

1838. YEAST PROTEIN WITH ANTIBIOTIC PROPERTIES. I. A. Parfentjev. Dept. of Microbiology, Yale Univ. School of Medicine, New Haven, Conn.

From alkaline watery extracts of brewer's and baker's yeasts, we isolated a complex protein with anti-infectious properties. Injected i.p. into the mice 12-24 hr prior to challenge, this protein protected them against infection by a number of organisms: Proteus, Salmonella, Pseudomonas; some protection against Brucella was reported by Kiser et al. 1956. Protecting dose is in the range of 0.02-0.2 mg of the yeast protein/mouse. Prophylactic injection of 5 mg of yeast protein/mouse made animals refractive to the Proteus infection for 1 month. Therapeutic effect of yeast protein on septicemia in rabbits and in pigeons was reported by Whitney and Arch, 1956. Fungicidal properties of yeast protein were described by Whitney et al. 1955. Desensitizing effect of yeast protein on mice sensitized by pertussis antigen was observed by Parfentjev, 1955. In vitro yeast protein has very little effect on bacteria. However, its application with soap was found beneficial for healing of skin lesions, particularly of mycetogenic origin, in dogs, Whitney et al., 1955. The complex yeast protein is soluble in water, not coagulable at 100°C, not dialyzable. It is precipitable at pH 2. In this protein we found organic phosphorus to which its biological activity can be related. The addition of cadmium precipitated the protein and blocked anti-infectious property of the new agent. The protein described is combined with ribonucleic acid while the latter by itself has very little, if any, antibacterial property, Parfentjev, 1953.

1839. PRODUCTION OF EXPERIMENTAL ENCEPHALOMYELITIS IN A NEW HOST, THE CAT. Philip Y. Paterson and Eugene D. Brand (intr. by A. E. Feller). Depts. of Microbiology and of Pharmacology, Univ. of Virginia, School of Medicine, Charlottesville.

Experimental encephalomyelitis has been produced in several species of laboratory animals by the injection of mammalian nervous tissue emulsified in Freund adjuvant. Work in our laboratories has shown that a new host, the cat, is also susceptible to experimental encephalomyelitis. Fourteen of 16 cats developed encephalopathy after receiving 1-8 injections of homologous nervous tissue in adjuvant at 2- or 3-wk intervals. Staggering gait and hindleg weakness or paralysis appeared in the 14 cats and progressed to complete quadriplegia in 4 animals. Dramatic pupillary inequality, nystagmus, and paresis of head and neck musculature were also observed. Five cats recovered from their initial encephalitic insult and subsequently suffered 1-4 neurological relapses. In 2 of these 5 animals severe neurological signs reappeared 77 and 126 days, respectively, after the final injection of nervous tissue. Histological changes of encephalomyelitis were demonstrable within the brain and spinal cord and consisted of intense vascular and perivascular cellular infiltrates. The vascular lesions on occasion were accompanied either by marked disorganization of adjacent

nervous tissue or by varying degrees of astrocytic proliferation. Six cats with encephalopathy developed circulating anti-brain antibodies; the remaining 10 animals failed to do so. Thus, the cat, which has been employed for decades for neurological research, can now be used for the study of experimental encephalomyelitis.

1840. FORMATION OF A STABLE CELL-VIRUS COMPLEX. Francis E. Payne and W. Wilbur Ackerman. Epidemiology Dept., School of Public Health, Univ. of Michigan, Ann Arbor.

Interaction of poliovirus (type I Mahoney strain) with monolayers of HeLa cells was studied by following the disappearance of virus from a reaction mixture and the rate at which cells develop the potentiality for cytopathology. An attempt was made to correlate the cytotoxic potency of a viral preparation with the multiplicities of viral input and exposure. By these techniques at least 2 reactions can be distinguished in the initiation of infection. The cell-virus complex resulting from the first of these is stable to dilution and possesses a potentiality for cytopathology which can be negated by antiviral immune serum. The product of the second reaction is insensitive to the action of immune serum. The kinetics of the first reaction will be considered in detail. The influences exerted on the rate of reaction by various concentrations of virus, various components of the medium and by varient lines of HeLa cells will be evaluated.

1841. FREE AMINO ACID POOL OF HUMAN CELLS IN TISSUE CULTURE. K. A. Piez* and H. Eagle. Natl. Insts. of Health, Bethesda, Md.

Free amino acid pools of several human cell lines grown on glass in a semi-defined medium have been examined by ion exchange chromatography. The concentrations of the individual amino acids were highly variable within any cell line, but the total accounted for about 25% of the TCA-soluble nitrogen and 2.5% of the total nitrogen. Ten of 13 nutritionally essential amino acids, supplied in the medium at approximately twice optimal levels, were concentrated by the cells up to approximately 10 fold. Cystine and arginine, however, could not be detected. Omission of any of the essential amino acids from the medium was reflected by a similar loss from the cell pool. Washing the cells on the glass with salt solution did not affect the cellular amino acid level, but disturbing the cells by scraping prior to the salt wash resulted in a rapid change in the composition of the pool. Equilibration between the amino acids in the pool, medium, and protein will be discussed.

1842. INHIBITION OF IMMUNE HEMOLYSIS BY PROTEINS AND PEPTIDES. O. J. Plescia, K. Amiraian* and G. Çavallo.* Inst. of Microbiology, Rutgers, The State Univ., New Brunswick, N. J.

Casein and egg albumin inhibited immune hemolysis and were split, as measured by an increase (~15%) in ninhydrin positive substances. The split products were probably large polypeptides, however, because they could be precipitated at a sufficiently high concentration of trichloroacetic acid (5%). This suggested proteolytic action in the immune system; accordingly the dipeptides glycyl-tyrosine, tyrosyl-glycine and phenylalanyl-phenylalanine were tried and found to inhibit (50%). They were split into their corresponding amino acids, identified chromatographically. The reaction of sensitized erythrocytes (EA) with complement (C') was inhibited more than the reaction of partially complemented erythrocytes (EAC'1, 2, 4) with C'3 (the 3rd component of C'). Incubation of C'3 with peptide at 37°C prior to the addition of EAC'1, 2, 4 had no effect on the extent of inhibition. This suggests that peptide is a substrate for one or more components already reacted with EA. Incubation of C' with peptide at 37°C prior to the addition of EA had no effect on the extent of inhibition. It appears, therefore, that C' must first be activated, probably by the immune system associated with EA. However, the presence of inhibitory concentrations of peptide (10^{-2} -10^{-3}M) caused a measurable decrease (~30%) in the titer of C'2 and in the fixation of C'2 during the preparation of EAC'1, 2, 4. Indole acetic acid, which inhibits peptidases, also inhibits immune hemolysis (~50%). On the basis of the above facts, it seems possible that proteolysis, probably of the cell wall proteins, plays a part in immune hemolysis and that peptidase action is in some way connected with C'2.

1843. SINGLE CELL PLATING TECHNIQUES APPLIED TO RADIOBIOLOGIC STUDIES IN MAMMALIAN CELLS. Theodore T. Puck. Dept. of Biophysics, Univ. of Colorado Med. Ctr., Denver.

Plating procedures have been developed in this laboratory whereby single mammalian cells multiply to form isolated, macroscopic colonies. The technique is simple and rapid, and completely analogous to the quantitative plating of bacteria in semi solid nutrient media. It permits determination of the number of cells capable of unlimited reproduction, the shape of the growth curve, and quantitative analysis of the action of growth inhibitors like toxic chemical agents, specific antibodies, and ionizing radiation. It also permits recognition and isolation of cell mutations which can serve as markers to illuminate genetic events in mammalian somatic cells (Puck, Marcus and Cieciura. J. Exper. Med. 103:273, 1956; Puck and Fisher. J. Exper. Med. 104:427, 1956). A variety of studies carried out with this technique have indicated that the primary action of high energy irradiation is to produce genetic damage to the somatic mammalian cell (Puck and Marcus. J. Exper. Med. 103:653, 1956). In the present paper these considerations are applied to furnish explanation of several of the noteworthy features of the acute, whole-body radiation syndrome in mammals.

1844. POLIOVIRUS SYNTHESIS IN MONKEY KIDNEY CELLS GROWN IN SYNTHETIC MEDIUM. Catherine Rappaport* and Joseph L. Melnick. Section of Preventive Medicine, Yale Univ., New Haven, Conn.

A synthetic medium is described for the outgrowth and proliferation of monkey kidney cells. Growth in this medium depends on defined chemical and physical conditions, including calcium and magnesium concentration and the surface of the glassware. Glycine may stimulate growth as early as 4 days after the cells have been removed from the monkey. Poliovirus may be synthesized in the absence of any exogenous sources of carbon, nitrogen, or energy. The synthesis of this virus thus represents efficient utilization of performed constituents of the cell. Only 2 supplements have been found to affect significantly the propagation of poliovirus in this system. Cysteine, or cystine, stimulates the rate of propagation and increases the yield of virus per cell. Clycine, which stimulates growth of the cell, inhibits the synthesis of the virus. It appears from these studies that the synthesis of poliovirus by monkey kidney cells is not an invariant consequence of the adsorption of the virus, but may be controlled by specific states of metabolism. A state favorable or unfavorable to virus synthesis may be induced by compounds which are physiological for the cell.

1845. ANTIBODY INACTIVATION OF TOBACCO MOSAIC VIRUS. I. Rappaport (intr. by Ray D. Owen). Botany Dept. Univ. of California, Los Angeles.

In the presence of an excess of rabbit antibody, the infectivity of dilute (5 x 10^{-4} mg/ml) TMV suspensions decreases rapidly until a stable level of survivors remains. The surviving virus particles are incorporated into aggregates, the average size of which is a function of the total concentration of both virus and antibody. It has been shown that inactivation results from the specific attachment of antibody to virus and is independent of the accompanying state of aggregation. Thus, viable virus particles exist within antibody-virus complexes despite the presence of excess free antibody in the medium. Moreover, the free antibody is not only capable of inactivating a fresh sample of TMV, but all other inactivation studies employing this partly absorbed antibody suggest no qualitative difference between its behavior and that of whole antiserum. If an additional aliquot of antiserum is added to antibody-virus complexes already in antibody excess, further inactivation occurs. The addition of large excesses of UV irradiated TMV or TMV protein subunits (both of which still retain their antigenic characteristics) to antibody-virus complexes, fails to reactivate any of the original infectivity. Antibody dissociated from the virus at pH 2.5 will reassociate after nautralization and inactivate the virus to the same extent as the controls. No irreversible damage to the virus by its combination with antibody has been detected. The data suggest that the antibody-TMV reaction is complex. The reaction progresses to a stable state which is characteristic of neither a reversible nor an irreversible system.

1846. CHARACTERISTICS OF AN ESTERASE DERIVED FROM PREPARATIONS OF THE FIRST COMPONENT OF COMPLEMENT. O. D. Ratnoff* and I. H. Lepow. Dept. of Medicine, School of Medicine, and Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.

Previous studies have demonstrated that an esterase could be derived from partially purified preparations of the first component of human complement and could also be eluted from antigen-antibody aggregates treated with human serum. The esterase digested p-toluene sulfonyl-L-arginine methyl ester and N-acetyl-L-tyrosine ethyl ester (Proc. Soc. Exper. Biol. & Med. 92:32, 111, 1956). In a large series of additional substrates tested, the esterase hydrolyzed only benzoyl-L-arginine methyl ester, N-acetyl-3, 5-dinitro-L-tyrosine ethyl ester, N-acetyl-L-phenylalanine ethyl ester and N-acetyl-L-tryptophane ethyl ester. No evidence of digestion of casein, gelatin, bovine serum albumin, human plasma or serum, denatured hemoglobin, edestin, lactalbumin, beta lactoglobulin, or human myometrium was obtained. A similar esterase was also found in preparations derived from rat and rabbit first component. The esterase digested N-acetyl-L-tyrosine ethyl ester optimally at 41°C and pH 7.3-8.4; the energy of activation calculated between 25° and 38°C was 12,000 calories/Ml. Human serum and complement-fixed serum contained a heat-labile inhibitor of the esterase, which was also inhibited by 10^{-3} M diisopropyl-fluorophosphate, by cysteine, reduced glutathione and thioglycollic acid, but not by pancreatic, soy bean or egg white trypsin inhibitors, heparin, ethylene diamine tetraacetate, ascorbate, peroxide or oxidized glutathione. Thus further evidence has been obtained first, of the identity of the enzyme derived from preparations of the first component of complement with that eluted from serum-treated antigen-antibody aggregates, and second, that this esterase cannot be identified with other known enzymes.

1847. GROWTH CURVE OF ROUS SARCOMA VIRUS (RSV) IN CHICK BRAIN. Frank J. Rauscher* and Vincent Groupé. Inst. of Microbiology, Rutgers, The State Univ., New Brunswick, N. J.

Previous studies in this laboratory showed that RSV propagated in the brain of newly-hatched chicks and killed them in relation to dose. When brains were removed daily from chicks inoculated intracerebrally with a 1/10 dilution of standard RSV, the resulting growth curve was found to be similar to that of many non-neoplastic animal viruses. First, virus disappears rapidly from the brain and 5 min after inoculation of RSV, virus was barely detectable in the brain. This was followed by a stationary phase of approximately 3 days during which the infective virus content of the brain remained low. Finally, beginning with the 4th day the virus content of the brain increased logarithmically and reached its maximum in moribund chicks on the 9th day. Further studies showed that the amount of virus recoverable from the brain was related to the infecting dose of virus. Thus, individual brains removed from moribund chicks previously inoculated with from 1 to 10,000 LD_{50} of RSV were found to contain appreciable and relatively uniform amounts of virus. However, when brains were removed from moribund chicks inoculated with less than one LD_{50} of RSV, no detectable virus at all was recovered in 4 of 5 instances. These data are in agreement with those of Bryan et al. on the yield of recoverable virus from tumor tissue and are at variance with common experiences with many non-neoplastic animal viruses.

1848. DOUBLE INFECTION OF CELLS WITH MEASLES AND POLIOVIRUS. Magdalena Reissig* and Joseph L. Melnick. Section of Preventive Medicine, Yale Univ., New Haven, Conn.

Infection of a cell with one virus may interfere with penetration and multiplication of another virus, or double infection of a cell with two viruses may occur. The latter had been previously observed when one of the infecting viruses produced intranuclear inclusions and the other cytoplasmic, but not when both inclusions had the same intracellular localization. The present results show that a cell may be infected simultaneously with two viruses each of which produces alterations in the nucleus and in the cytoplasma. Cultures of monkey kidney epithelial cells infected with, and actively producing, measles virus are fully susceptible to poliovirus. In such doubly infected cultures, mononucleated and multinucleated cells may contain, not only the intranuclear and cytoplasmic inclusions characteristic of measles virus infection, but also intranuclear inclusions and paranuclear eosinophilic masses typical of poliovirus infected cells. Thus in the same nucleus there were often present a large, type A eosinophilic inclusion characteristic of infection by measles virus, and several smaller and darker inclusions typical of poliovirus infection. When cultures previously infected with measles virus in an advanced stage of cytopathic degeneration, were challenged with poliovirus, no significant difference was found in their poliovirus yields, as compared with those of cultures infected with poliovirus alone.

1849. DELAY IN APPEARANCE OF PLAQUES WITH PARTIALLY INACTIVATED POLIOMYELITIS VIRUS. W. A. Rightsel,* I. W. McLean, Jr., E. A. Timm,* P. Schultz* and A. R. Taylor.

Research Labs. Parke, Davis & Co., Detroit, Mich.

Characteristics and specificity of the previously reported phenomenon (Timm et al. Fed. Proc. 15:618, 1956) Taylor et al. Ibid. 15:617, 1956; Beardmore et al. Ibid. 15:611, 1956) of delayed cytopathogenicity of formalin treated poliomyelitis virus have been further investigated by means of the plaque technique (Dulbecco and Vogt. J. Exper. Med. 99:167, 1954). Tissue culture suspensions of Mahoney, MEF-1, and Saukett strains of poliomyelitis virus were treated with formalin, Wescodyne (West Disinfecting Co., Long Island City, N. Y.), ultraviolet irradiation, and by heat for varying periods of time. Aliquots of the partially inactivated virus were removed at intervals and the rate of appearance of plaques on monolayers of trypsin-dispersed monkey kidney cells was determined. Dilutions of untreated virus were used for comparison. Also, the inactivation rates were followed with each type of poliomyelitis virus by the plaque technique. The appearance of plaques was consistently delayed with formalin-treated virus. This phenomenon was also observed when the formalin was removed by dialysis and was more prolonged than when the formalin was neutralized by treatment with damidone. The delay was not manifested with virus altered by the other methods of treatment listed above, and, hence, appears to be unique to formalin. The interval until the appearance of plaques with formalin-altered virus was dependent on the length of time the sample was allowed to stand before testing. The conflicting results obtained in formalin inactivation may be associated with and explained in part by this unique effect of formalin on the ability of poliomyelitis virus to initiate infection of tissue culture cells.

1850. FRACTIONATION AND ALTERATION OF COMPLEMENT-FIXING POLIOVIRUS ANTIGENS. B. Roizman,* H. J. Rapp* and M. M. Mayer. Dept. of Microbiology, Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, Md.

Depending on length of storage or other variables in the process of production at least two complement fixing poliovirus antigens may be present in infected tissue culture fluid. The "acute" antigen fixes complement with sera collected soon after onset of disease and appears to have a broader specificity than the "convalescent" antigen which reacts with sera taken during the convalescent phase. Purification studies with type 1 (Mahoney) indicate that these antigens may be separated by ultracentrifugation in a sucrose density gradient. The "acute" antigen lacks infectivity, and has a lower sedimentation rate than the infectious virus. The "convalescent" antigen contains the bulk of infectious particles. Furthermore, purified infectious virus which shows "convalescent" antigen activity may be converted to "acute antigen" by U. V. inactivation or heating. It is suggested that the early antibody response in patients may be directed against a virus degradation product or incomplete virus. (Aided by a grant from the Natl. Fndn. for Infantile Paralysis, Inc.)

1851. PRODUCTION OF ANTIBODIES AGAINST RABBIT THYROID IN RABBITS BY INJECTION OF FOREIGN THYROID EXTRACTS. Noel R. Rose and Ernest Witebsky. Dept. of Bacteriology and Immunology, Univ. of Buffalo School of Medicine, Buffalo, N. Y.

Injection of rabbit thyroid extracts with Freund adjuvants into rabbits leads to the formation of specific thyroid antibodies reacting with rabbit thyroid extract and also capable of cross-reacting with thyroid extracts of certain other species such as beef and hogs. Conversely, injection of beef or hog thyroid extracts into rabbits not only elicits antibodies directed against the extract used for injection, but also antibodies capable of reacting with rabbit thyroid extracts. In this latter instance, the rabbit thyroid antibodies have been demonstrated by tanned cell hemagglutination, but not so far by complement fixation. Absorption of hog or beef thyroid antisera with rabbit thyroid extract removes the reaction with rabbit thyroid extract but not with the homologous antigen. Absorption of anti-rabbit thyroid rabbit sera with foreign thyroid extracts removes the ability of the rabbit serum to react with foreign thyroids, but does not remove the reaction with the homologous rabbit thyroid extract. (Supported in part by a research grant, C 2357, from the Natl. Cancer Inst., Natl. Insts. of Health, PHS.)

1852. PROCEDURE FOR INCREASING SENSITIVITY OF TESTS FOR SPECIFIC TREPONEMAL ANTIBODY. Barbara J. Rosenau* and John F. Kent. Dept. of Serology, Walter Reed Army Inst. of Research, Washington, D. C.

Percentage of Treponema pallidum specifically immobilized, or rendered methylene blue resistant, by antiserum and complement (C') increases with time, but C' deterioration at the optimal reaction temperature (35°C) limits incubation to 18 hr. Supplements of C' however permitted extending the incubation period with resultant increase in sensitivity for treponemal antibody. In practice, anaerobic incubation was interrupted at 18 hr and aliquants (0.05 ml) of active and heat-inactivated guinea-pig serum added to tests and controls; anaerobiosis was then reestablished and incubation continued to 40 hr. Syphilitic rabbit sera titrated by the standard (TP_{18}) and experimental (TP_{40}) techniques showed 2- to 4-fold increases in titer. Human syphilitic sera selected on the basis of a negative or weak reaction at 18 hr were similarly tested. Eighteen found negative in TP_{18} tests proved weakly-reactive or reactive in 40-hr tests; 6 found weakly-reactive at 18 hr were reactive in TP_{40} tests. To determine whether the increase in sensitivity was associated with loss in specificity for treponemal infection, TP_{40} tests were also conducted with sera of nonsyphilitic persons found negative in TP_{18} tests. In a group of 49 specimens, including 17 which reacted with cardiolipin antiques, 1 proved weakly-reactive at 40 hr. The TP_{40} procedure is discussed with respect to its potentialities for increasing the value of treponemal immobilization and methylene blue tests in diagnosis, and in differentiating persons with latent syphilis from those whose sera react adventitiously with cardiolipin antigens.

1853. ANTIBODY PRODUCTION DEMONSTRATED BY PASSIVE TRANSFER OF CELLS AND DYE LOCALIZATION. L. T. Rosenberg,* M. H. Chandler,* A. S. Gordon and E. E. Fischel. Dept. of Biology, Grad. School of Arts and Science, New York Univ., and Dept. of Medicine, Bronx Hosp., New York City.

Technique of passive cutaneous anaphylaxis (Ovary) has been applied to the detection of antibody produced by donor spleen cells in the skin of recipient guinea pigs. Recipients were challenged i.v. with antigen and Evans Blue Dye at varying intervals after cell transfer. Counts indicated that 10^5-10^7 nucleated cells were injected. This number is capable of specifically sensitizing recipients at the sites of injection. When spleen cells are transferred from donors 1-7 days after a single immunizing dose, challenge results in dye localization at the cell site. With transfer at longer intervals

after immunization, dye localization also occurs at the site of injection of the supernate of the first cell wash. With transfer at still longer intervals, the supernate of the first cell wash alone appears to confer sensitivity. The sites of heated cells were negative. The sites of injection of frozen and thawed cells were infrequently positive. It appears that sensitization of recipients is demonstrable for only a few days. There is no evidence of systemic sensitization of recipient animals. This method may facilitate the quantitative study of the production of antibody by small numbers of various cell types.

1854. ENDOCRINE AND LIPID ASPECTS OF EXPERIMENTAL ATHEROSCLEROSIS IN DOGS. S. Rosenfeld,* J. Marmorston, H. Sobel,* J. Mehl, J. Lewis* and A. White.* Inst. for Med. Research, Cedars of Lebanon Hosp., Depts. of Medicine and of Biochemistry and Nutrition, Univ. of Southern California, Los Angeles.

Fifty-eight dogs (33 beagles and 25 mongrels) were divided into the following groups and studied for from 2 to 12 months: a) Control (2-12 months). b) Thiouracil feeding (10 months). c) Thyroidectomy (6 months). d) Thiouracil, plus cholesterol feeding (10 months). e) Thyroidectomy, plus cholesterol feeding (6 months) f) ACTH, 40 U 1-3 doses, followed in 2-4 months by thiouracil and cholesterol feeding (6 months). Serum cholesterol, phospholipid and alpha and beta lipoprotein determinations were made at monthly intervals. The average serum cholesterol levels observed during the course of the experiments were as follows: In the control dog, 150-200 mg%. In thiouracil fed, or thyroidectomized dogs, 300-400 mg%. Thiouracil plus cholesterol feeding induced a rise to 500-700 mg%. Thyroidectomy plus cholesterol feeding produced a rise to 900 mg% in male dogs, and 550 mg% in female dogs. In dogs pretreated with ACTH and then fed thiouracil and cholesterol, the levels rose to an average of 1400 mg%. It is concluded that: 1) Thiouracil feeding is less effective than thyroidectomy in producing hypercholesterolemia in cholesterol fed dogs. 2) Thyroidectomized dogs fed a high cholesterol diet appear to show a male-female difference in susceptibility to the development of hypercholesterolemia. 3) Pretreatment with ACTH greatly enhances the hypercholesterolemia in thiouracil plus cholesterol fed dogs. (Supported in part by California Fndn. for Med. Research and PHS Grant No. H-2199).

1855. TREATMENT OF RED BLOOD CELLS WITH ALKALOIDS AND THEIR AGGLUTINATION BY TANNIC ACID. Karl L. Roth. Southern Div., Albert Einstein Med. Ctr., Philadelphia, Pa.

Experiments with the hemolytic system of Wunderly (Helvet. Physiol. et pharmacol. acta 5:91, 1947) who used reduced glutathione for lysis of RBC pretreated with Na-Desoxycholate, led to the investigation of other low molecular weight substances with the purpose of producing an agglutinating system, where both the (presumably adsorbed) "antigen" and the "antibody" are known, can be measured, and their molecular sizes related to the degree of the reaction. Ten μg/ml each of neostygmine, hyoscin, caffeine, strychnin, morphine and pilocarpine were incubated for 30 min/37°C with buffered 2% RBC suspensions (0.2 ml). No hemolysis, agglutination, or apparent damage to cell integrity resulted. After 3 washes with buffer (pH 7.2) an equal volume of m-digallic (tannic) acid -0.1 mg/ml - was added and the suspensions reincubated. Visible agglutination occured in all samples in less than 5 min. Normal RBC remained unaffected by this concentration of tannic acid. Reaction could be inhibited by (keeping or) adding free alkaloid ("antigen") to the pretreated RBC before reincubation. Reversing the order of mixing, i.e.: incubating RBC with tannic acid, washing, and adding alkaloids, did not result in agglutination, although the cells were sensitized, as shown by their hemolysis in fresh serum. These experiments may be helpful to establish a simple model for agglutination by Coombs' sera of RBC coated with incomplete agglutinins and to determine the minimal forces necessary to reach the endpoint of an agglutinin titer. (Supported by Grant H-1919 from the Natl. Insts. of Health and by a Fellowship from the J. I. Wagman Memorial Fndn., Philadelphia, Pa.)

1856. RELATIONSHIP OF AGGLUTINOGEN TO OTHER ANTIGENS OF H. PERTUSSIS. L. F. Schuchardt,* J. F. Sagin* and J. Munoz. Merck Inst. for Therapeutic Research, Rahway, N. J.

Role of H. pertussis agglutinogen and its relationship to the protecting and hemagglutinating antigens, the histamine sensitizing substance, and the heat-labile endotoxin have not been clearly determined. Although other workers have made purified preparations of agglutinogen, the methods used were rather harsh and the immunological homogeneity of these fractions was not definitely established. An immunologically homogeneous form of agglutinogen has been obtained by precipitation with either Na_2SO_4 or $(NH_4)_2SO_4$ from supernatants of H. pertussis cells disrupted by sonic disintegration. This preparation was found to contain essentially all of the agglutinogen activity of the original cells and only about 0.4% of the total nitrogen. The fraction was nontoxic and failed to sensitize mice to histamine. Although these preparations did not protect mice challenged with virulent H. pertussis and did not agglutinate chicken red blood cells, they were shown to be antigenic since mice and rabbits injected with these fractions developed specific agglutinins for H. pertussis cells. Immunologic homogeneity was established by the serum agar diffusion techniques of Oudin and Ouchterlony. These observations clearly demonstrate that H. pertussis agglutinogen is a specific substance distinct from the substance or substances responsible for protection, histamine sensitization, toxicity and humagglutination.

1857. A NEW SEROLOGICAL TEST FOR THE DETECTION OF VIRAL ANTIGENS. Diego Segre (intr. by D. T. Berman). Dept. of Veterinary Science, Univ. of Wisconsin, Madison.

Antibody (gamma globulin from the serum of hyperimmunized animals) was adsorbed on particles of the anion exchanger, Amberlite IRA-400. Such antibody-coated particles were then agglutinated on addition of materials containing the specific viral antigen. Viral antibodies were also detected in convalescent serums by means of an inhibition reaction in which dilutions of the test serum were premixed with amounts of virus known to cause agglutination. The test was applied to the viruses of hog cholera and bovine vesicular stomatitis. Hog cholera virus was detected in the serum of experimentally infected pigs, from 3 to 6 days after exposure, and in the spleen and kidney of the same pigs 7 days after exposure. The serums from 4 pigs involved in an outbreak of hog cholera were tested. Three serums were found positive, one negative. Hog cholera virus was detected in spleen and kidney of 1 pig from the same herd, and in a commercial preparation of hog cholera virus (whole infected blood). Vesicular stomatitis virus was detected in several pools of allantoic fluids from infected chicken embryos. Resin particles coated with antibody to the New Jersey type of vesicular

stomatitis virus were agglutinated by both New Jersey and Indiana type viruses. Antibodies were detected by the agglutination-inhibition test in immune hog cholera and vesicular stomatitis serums. It is hoped that the test described will be applicable to many more antigen-antibody systems.

1858. COMPARISON OF GROWTH OF CERTAIN MYCOBACTERIA IN HeLa, MONKEY KIDNEY, HUMAN AMNION AND X-IRRADIATED HeLa CELLS. Charles C. Shepard. Virus and Rickettsia Section, Communicable Disease Ctr., Public Health Service, Montgomery, Ala.

In an attempt to elucidate properties of different tissue culture cells that might influence the ability to mycobacteria to grow in them, a comparison was made of HeLa, monkey kidney, and human amnion cells in monolayer. Mycobacteria included human tubercle bacilli of full and reduced virulence, and several other species of human pathogenic reputation, such as M. fortuitum, the "yellow bacillus," and M. balnei. Appropriate media were worked out for the infection of the cells by promoting phagocytosis of the mycobacteria, and following their infection the cells were kept in media providing optimal nutrition. In general it was found that the order of growth rates of different mycobacterial strains observed in HeLa cells was confirmed in the other types of cells. In monkey kidney cells the growth rate of each strain was roughly the same as in HeLa cells, but estimations were more difficult due to the variation in cell types. In amnion cells growth rates of all mycobacteria were roughly half of that seen in HeLa. Characteristic mycobacterial growth patterns were not fully developed in amnion cells, e.g., the virulent tubercle bacilli were not easily differentiated from strains of reduced virulence. Giant HeLa cells produced by X-irradiation (Puck) allowed admirable development of characteristic mycobacterial patterns, but the mycobacterial growth rates in them were not apparently different from that observed in nonirradiated cells.

1859. THYRALBUMIN—A NEW ANTIGEN FROM THYROID TISSUE. Sidney Shulman,* Noel R. Rose and Ernest Witebsky. Dept. of Bacteriology and Immunology, Univ. of Buffalo, School of Medicine, Buffalo, N. Y.

An albumin fraction of hog thyroid extract has been prepared and characterized. The product, designated RP 2.50-3.00, is obtained by precipitation with ammonium sulfate between the indicated levels of molarity. It is a red-brown material with a single absorption maximum in the visible region, located at 4080 Å. It shows a single peak in the ultracentrifuge, corresponding to the slowest component (peak V (Sidney Shulman, Noel R. Rose and Ernest Witebsky. J. Immunol. 75:291, 1955)), in crude saline extract. Serological comparisons indicate considerable antigenic similarity to blood serum itself, although physicochemical data show that a simple identification with serum albumin or with hemoglobin cannot be made. The further characterization of this material as a species-specific tissue antigen will be described. (Supported in part by a research grant, C2357, from the Natl. Cancer Inst., Natl. Insts. of Health, PHS.)

1860. APPLICATIONS OF CONTRASTING FLUORESCENT LABELS FOR TWO ANTIBODIES. Arthur M. Silverstein and Paul J. Berrigan (intr. by Fred D. Maurer). Armed Forces Inst. of Pathology, Washington, D. C.

Method of Coons and Kaplan (J. Exper. Med. 91:1, 1950) for the preparation of fluorescein-labeled antibodies has found broad application to the study of the distribution of various antigenic materials in vivo. It has also been applied to the study of the sites of antibody production (Coons et al. J. Exper. Med. 102: 49, 1955) and to the rapid detection and identification of bacteria on smears (Moody et al. J. Bact. 72:357, 1956). The need for a second fluorescent label for antibodies to be used in conjunction with the yellow-green fluorescein label has been felt for some time. This label should have a contrasting color, so that each label can be visualized separately when the two are used in conjunction in the same field of view, or in the same cell. A method for specific staining of two antigenic components simultaneously might have several applications: it would simplify the establishment of a schema for the rapid identification of micro-organisms; it would allow a study of the distribution and effects of two antigenic components in vivo, and of the antibody response evoked by the several antigens. Preliminary studies (Silverstein. J. Histochem. In press) have indicated that an amino derivative of Rhodamine B might satisfy these requirements. This fluorochrome emits a bright orange fluorescence in ultraviolet light, in marked contrast to the yellow-green of fluorescein. Its conjugation to antibody is accomplished in a manner analogous to that of fluorescein. The technical aspects of the preparation and use of the contrasting fluorescent label will be discussed, and the results of preliminary applications to the differential staining of bacterial smears and of antigens in tissue will be presented.

1861. INHIBITION OF ANTIBODY RESPONSE TO HETEROLOGOUS PROTEIN IN RABBITS BY NEONATAL EXPOSURE. Richard T. Smith, R. A. Bridges, E. M. Thomson, H. W. Kearnes, V. Olmanson and M. L. Will (intr. by Lewis Thomas). Univ. of Minnesota, Minneapolis.

In these experiments a single i.p. injection or oral dose of crystalline bovine albumin (BSA) on the day of birth in rabbits resulted in consistent failure to produce specific precipitating or complement fixing antibody in response to subsequent BSA challenge. For example, of rabbits which received 10-200 mg i.p., only 4 of 65 produced antibody at 100 days of age; 0 of 21 at 200 days; and 1 of 12 at 300 days. Between 330 and 390 days, 14 of 28 survivors produced antibody for the 1st time. In contrast 7 of 9 rabbits receiving less than 1.0 mg BSA, and 21 of 22 controls produced antibody at 100 days. In similar experiments, 13 of 47 rabbits which received 100 mg BSA on the 3rd day of life, 1 of 4 on the 9th day, and 7 of 16 on the 15th day of life developed precipitins at 100 days. By 380 days only 1 of the 15-day group was still unresponsive. Antigen clearance was prolonged in unresponsive rabbits, half-life values averaging 6.5 days. Rabbits not responding to BSA developed antibody to egg albumin. Unresponsive rabbits temporarily produced specific antibody when given lymph node cells from immune animals. Delayed hypersensitivity to BSA without detectable antibody formation was induced in unresponsive rabbits by intradermal injection of specific BSA precipitates. The bearing of these data upon the mechanism of "acquired tolerance" will be discussed. (Aided by Natl. Insts. of Health grant H-2093.)

1862. ENZYME TREATED ERYTHROCYTES IN DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS. Georg F. Springer and Marianne D. Hayes.* William Pepper Lab. Univ. of Pennsylvania, Philadelphia.

In 1946 Burnet and Anderson (Brit. J. Exper. Path. 27:236) reported that erythrocytes may be altered by preparations of Newcastle disease virus, pointing to a new approach in diagnosis and possibly elucidation of

etiology of infectious mononucleosis. While studies were in progress in our laboratory, Wöllner developed a test for this disease by treating sheep erythrocytes with papain (Ztschr. Immunitätsforsch. 112:290, 1955). He considers his test to be specific for infectious mononucleosis. We have adopted a modification of this procedure and obtained specific reproducible results. The test was found to be reliable in that it gave unequivocal results, even if the titers were low. False positives have thus far not been observed. While we have been able to inactivate the receptor for infectious mononucleosis with papain preparations, this has not yet been possible with trypsin. Data obtained with different preparations of proteolytic enzymes on erythrocytes from different species will be presented. Attempts to specifically inhibit red cell agglutination by sera from patients with infectious mononucleosis will be discussed. There is indication that at least part of the receptor for infectious mononucleosis antibody is not destroyed by enzymatic action, but released into the supernatant fluid. Serum sickness and Forssman receptors were not removed from the erythrocyte surface by these procedures.

1863. ALTERATION OF BACTERIAL ENDOTOXINS BY HUMAN AND RABBIT SERUM. John E. Stauch and Arthur G. Johnson (intr. by W. J. Nungester). Dept. of Bacteriology, Univ. of Michigan, Ann Arbor.

A fraction of purified Salmonella typhosa endotoxin sedimenting on 2 hr centrifugation at 10,000 g was incubated in serum devoid of specific antibody at 37°C for various time intervals. Following centrifugation of the serum-endotoxin mixtures at 35,000 g for 2 hr, the pellet and supernate were assayed for endotoxin by the quantitative precipitin technique. Forty to fifty percent of endotoxin (as measured by precipitating activity) was lost following incubation in rabbit serum, while 100% of this lipopolysaccharide was recovered following incubation in saline. A supernate/pellet ratio in rabbit serum of approximately 3 contrasted with a ratio of 0.1 following saline incubation. The magnitude of loss was a function of incubation time, with a maximum occuring at 4 hr. Incubation of serum-endotoxin at 4°C resulted in essentially complete recovery of endotoxin. Sixty to seventy percent of this lipopolysaccharide was recoverable from heat-treated serum (56°for 30 min), whereas no endotoxin was detectable following incubation in serum at 65°C for 6 hr. Serum treated with versene, resin, or zymosan showed no diminution of ability to alter endotoxin. Addition of 0.1 M mercuric ions, or incubation in serum from rabbits tolerant to Serratia marcescens endotoxin resulted in 100% recovery of endotoxin. The foregoing results were essentially similar in tests employing serum from 14 human volunteers, with optimum conditions (incubation at 37°C for 6 hr at pH 8) resulting in recovery of only 25% of endotoxin. Preliminary fractionation studies indicated that activity is confined to that fraction of serum precipitable by 50% ammonium sulfate.

1864. DEVELOPMENT OF PLATELET ANTIBODIES. Mario Stefanini and Sten A. Kistner.* Joseph Stanton Labs., Saint Elizabeth's Hosp., and Dept. of Medicine, Tufts Univ. School of Medicine, Boston, Mass.

Antigenic structure of human and rabbit platelets was presumably modified by treatment with Newcastle virus, bacterial filtrates (from Streptococci and Staphylococci) and various drugs (aminopyrine, quinine, quinidine). Treated platelets were injected in 3 groups of animals, 1 receiving human platelets (hetero-immunization), 1 receiving platelets from normal rabbits (iso-immunization) and a 3rd group receiving their own modified platelets (? auto-immunization). Development of platelet antibodies was evaluated by standard immunologic techniques. Results: a) high titer in vitro platelet agglutinins developed in the hetero-immune group, without evidence of specific antigenicity of modified versus nonmodified platelets. In 1 instance the titer of a naturally occurring iso-antibody decreased as a hetero-antibody developed. Iso-immunized animals did not develop antibodies demonstrable in vitro. Auto-immunized animals often developed both auto- and iso-antibodies demonstrable in vitro, both in the presence or absence of the specific modifying agent. b) Injection of the specific modifying agent in auto- and iso-immunized animals, however, was followed by significant thrombocytopenia when evidence of immunization was found in vitro. Status of immunization was short-lived; c) the electrophoretic pattern of proteins and glycoproteins in the serum of auto- and iso-immunized animals often shows changes similar to those observed in other instances of experimental immunization. Conclusions: 1) iso- and auto-immunization against platelets developed often in rabbits receiving their own modified platelets; 2) many of the experimental results were quite consistent with findings in idiopathic thrombocytopenic purpura in humans; they could, perhaps, be applied to the interpretation of the pathogenesis of thrombocytopenic states in man.

1865. LEUKOAGGLUTINATION: RELATION OF MONOCYTIC LEUKEMINA, PLASMA CELL MYELOMA AND THYMOMA TO LEUKEMIA AND NEOPLASIA. Bernhard Steinberg and Ruth Martin.* Toledo Hosp. Inst. of Med. Research, Toledo, Ohio.

Previous studies established that agglutinins can be produced against specific types of leukocytes. The process was defined as, "clumping of leukocytes, either circulatory or fixed by antibodies." It was shown that neoplastic lymphocytes (lymphosarcoma) differ antigenically from normal and leukemia lymphocytes. Normal granulocytes are antigenically similar to leukemic. The present study is extended to the antigenic relationship of monocytes from monocytic leukemia, plasma cells from plasmoma, and thymic cells from thymoma, to each other and to neoplastic cells, lymphocytes and granulocytes.

1866. VERY RAPID FORMATION OF ANTIBODY IN VITRO. Kingsley M. Stevens and John M. McKenna.* Dept. of Virology, Merck Sharp and Dohme Research Labs., West Point, Pa.

In a previous paper (J. Immunol. in press), antibody formation was demonstrated within 4 hr after in vivo introduction of bovine-γ-globulin (BGG). Using the completely in vitro technique just described (Fed. Proc. 16: 1957), a study was made of the antibody found in spleen extracts following incubation for 1 hr at 37°C. The mean hemagglutinating titer after incubation with 500 μg/ml of BGG was 32 while the mean control titer was 8. This difference is significant ($P < 0.001$). The titers found after BGG treatment could be specifically neutralized with BGG. When BGG was added but incubation was omitted or carried out at 4°, titers did not differ from controls. When entire spleens were extracted in a small volume following incubation, titers up to 256 were secured. On normalization to a unit basis, this gives 815 HA units/gm spleen for the experimental sample vs. 140 HA units/gm for the control. It appears that in this completely in vitro system, where cell replication does not occur, a small amount of antibody is produced almost immediately but little, if any, is formed later. Certain theoretical implications will be discussed.

1867. ACCESSORY FACTORS IN RABBIT AND HUMAN BLOOD INVOLVED IN BACTERICIDAL TEST FOR TYPE-SPECIFIC ANTIBODY TO GROUP A STREPTOCOCCI. G. H. Stollerman, F. S. Kantor and B. D. Gordon (intr. by Samuel Feinberg). Dept. of Medicine, Northwestern Univ., Chicago, Ill.

Previously only human and monkey blood were reported to contain all of the accessory factors necessary for the bactericidal test for type-specific antibody to group A Streptococci. The whole blood of most rabbits immunized in our laboratory, however, developed strong type-specific bactericidal activity. Addition of rabbit antisera to the blood of certain normal rabbits (the indirect test) also resulted in strongly positive tests. Addition of human type-specific antibody (serum or gamma globulin) to rabbit blood was not bactericidal. Certain rabbit antisera were found to be nonbactericidal with all normal rabbit bloods tested. In most instances, however, rabbit antisera were strongly bactericidal when added to the blood of a suitable normal rabbit. Individual normal rabbits of the strain studied (a cross of Flemish bucks and either New Zealand or German Checker does) varied markedly in the ability of their blood to activate the system. Although most human bloods were suitable, some were relatively ineffective in producing bactericidal activity upon the addition of rabbit or human antisera. These differences were not explained adequately by variations in the hemolytic titer of serum complement, although the heat-labile components of the latter are required for this opsono-phagocytic system. Treatment of human serum with zymosan under conditions that remove properdin resulted in virtually complete loss of bactericidal activity. The possible removal of other serum factors by this procedure was not excluded.

1868. ANAPHYLAXIS IN THE MOUSE. Sanford H. Stone and Jules Freund. Public Health Research Inst. of the City of New York, New York City.

Mice injected with antigens incorporated in paraffin oil adjuvants containing mycobacteria produced amounts of circulating antibody comparable to those obtained in rabbits immunized by similar procedures. Antibody nitrogen in mouse antisera was frequently over 1 mg/ml. Fatal anaphylactic shock was elicited in mice passively sensitized by such antisera. Two i.p. injections of antigens (i.e. human serum albumin, horse serum, horse serum globulins) combined with complete adjuvants sensitize mice so that severe or fatal anaphylactic shock is regularly elicited after injection of the homologous antigen into the lip or foot pad, as well as by the i.v. route. Over 50% mortality may be obtained by injection of the challenging dose into the lip or foot pad. Systemic anaphylaxis inhibited local inflammatory reactions at the site of injection. Mice sensitized to both busycon hemocyanin and human serum albumin were given hemocyanin intralabially and human serum albumin intravenously. There was little or no labial reaction. Mice similarly sensitized injected intralabially only reacted to hemocyanin with intense local Arthus reactions. Serum antibody was measured from individual mice. Blood from the cut tail was diluted in a WBC pipette. Normal mice injected intralabially with dilutions of mouse antiserum were later injected with homologous antigen and Evans blue i.v. The dye colored the lip containing antibody. Antibody nitrogen was calculated by comparison with a pool of mouse antiserum of known antibody nitrogen content.

1869. A NEW METHOD FOR THE PURIFICATION OF PRECIPITATING AND NONPRECIPITATING ANTOBODY. G. Bonar Sutherland* and Dan H. Campbell. Dept. of Physiology, Univ. of Kansas, Lawrence, and Dept. of Chemistry, California Inst. of Technology, Pasadena.

Preliminary studies with such proteins as ovalbumin, bovine serum albumin or gamma globulin and hemocyanin, showed that they were adsorbed to glass surfaces strongly enough to resist removal by many washings of 0.9% saline. Such antigen-coated glass surfaces could then be used for the detection of antibody or isolation of antibody by dissociation and elution at pH 3.0. Under such conditions the antigen remains adsorbed to the glass surface and the adsorbent can then be used again. A typical preparation of rabbit antiovalbumin antibody was made as follows; a solution of 0.1% ovalbumin in 0.9% saline was poured into a glass column (vol. about 1 lt) filled with fine glass beads (av. diameter of 44 microns). The beads were allowed to remain in contact with the solution for 24 hr and then thoroughly washed with saline. Rabbit antiovalbumin serum, diluted to contain about 0.5 mg of precipitating antibody protein per milliliter was added to the column and allowed to stand for 24 hr. The beads were again thoroughly washed with saline and then treated with glycine-HCl buffered saline at pH 3.1. The acid eluate was adjusted to pH 8.0 and then concentrated by pervaporation to a convenient volume. The preparation contained 80% precipitable antibody. The remaining 20% was gamma globulin which was found to specifically combine with ovalbumin, but did not precipitate. It is suggested that the 20% material was a non-precipitating antibody.

1870. ISOLATION OF NUTRITIONAL VARIANTS FROM A MAMMALIAN CELL CULTURE. H. E. Swim* and R. F. Parker. Dept. of Microbiology, Western Reserve Univ., Cleveland, Ohio.

During the course of studies on the nutritional requirements of human fibroblasts, strain U12-705, it was observed that in certain instances the cultures gradually became able to proliferate in media which appeared to be inadequate initially. To examine the possibility that the culture contains more than 1 nutritional cell-type after 3 yr in vitro, cells which are propagated in medium 705 (5% chick embryo extract, 20% horse serum, 75% solution 703) were placed in media lacking either serum (63) or embryo extract (704). During the 1st wk the fibroblasts proliferated at a moderate rate in both experimental media, but thereafter gradually degenerated until all the cells appeared to be involved. Subsequently a few healthy cells appeared in the cultures and their progeny (U12-63 and U12-704) continue to proliferate at rates comparable to that of U12-705 strain. A third subline (U12-79) derived from U12-63 requires 8 vitamins including choline and i-inositol. By a similar procedure, 2 variants of U12-79 were isolated which do not require choline (U12-82) or i-inositol (U12-80). The relative distribution of U12-80 cells in a population of U12-79 is approximately 1 to 10^6. In this instances, it would be impractical to isolate U12-80 as single cells selected at random from the population. The application of the selection method to the study of the genetics of somatic cells cultured in vitro will be discussed and the nutritional and cultural characteristics of the variants isolated from U12-705 will be compared.

1871. ENHANCEMENT OF VIRUS MULTIPLICATION. Igor Tamm and Marjorie M. Nemes.* Rockefeller Inst., New York City.

5-Methyl-2-D-ribobenzimidazole markedly increases the yield of influenza B virus in the chorioallantoic membrane (CAM) in vitro (Tamm. Virology 2: 517, 1956). In

membranes from 8- to 13-day embryonated eggs the yield of virus in untreated cultures decreased with increasing age of eggs, whereas the degree of enhancement in the presence of 5-methyl-2-D-ribobenzimidazole increased. In effect this compound restored the diminished capacity of chorioallantoic membrane from older eggs to produce virus. Supplementation of the medium of buffered salts and glucose with a large number of vitamins, amino acids, purines, pyrimidines and other known metabolites did not increase the yield of influenza virus. The presence of these metabolites also did not affect enhancement by 5-methyl-2-D-ribobenzimidazole. Omission of glucose from the simple medium resulted in marked reduction in the amount of virus produced. Addition of 5-methyl-2-D-ribobenzimidazole to the glucose-free medium still increased the yield of virus. However, the amount of virus produced was less than that produced in the presence of glucose. Supplementation of the simple medium with horse serum moderately reduced the yield; calf and rabbit sera caused marked reduction, whereas chicken serum caused a striking increase. The enhancing factor in chicken serum was nondialyzable and stable at 50°C for 30 min. At concentrations causing increased yield of virus 5-methyl-2-D-ribobenzimidazole did not increase oxygen uptake, incorporation of adenosine-8-^{14}C into ribonucleic acid, or cellularity of CAM. This compound showed no enhancing activity on the multiplication of vaccinia and poliomyelitis viruses.

1872. EFFECTS OF INTRAPERITONEAL INJECTIONS OF LARGE DOSES OF BOVINE SERUM ALBUMIN AT HATCHING ON SUBSEQUENT INOCULATIONS OF HOMOLOGOUS AND HETEROLOGOUS PROTEINS. C. Tempelis,* and H. R. Wolfe and A. Mueller.* Dept. of Zoology, Univ. of Wisconsin, Madison.

Arbor Acre White Rocks and White Leghorn males were injected with large doses of bovine serum albumin 8-40 hr after hatching. Groups of these birds were reinjected at 6, 12 and 22 wk of age with 40 mg/kg body weight of the homologous antigen or human gamma globulin and bled at different intervals after the inoculation. The disappearance of antigen from the circulation was similar in the experimental (injected at hatching) and control animals that were not injected at hatching. The antibody nitrogen of the circulating blood was determined. At 6 wk of age the response of the experimental group was much reduced and a number of animals did not produce detectable antibodies. The 12-wk birds showed a lower response than the controls but the effect of the early inoculation was not as striking as shown by the 6-wk animals. One 22-wk group of experimental birds that was injected at 30 hr of age responded as well as the controls but another group that received a slightly smaller dosage at 8 hr of age did show significant differences. One group was inoculated with human gamma globulin at 6, 12 and 22 wk of age and there was no difference in the response of the controls or those that received BSA at hatching. Over 500 animals were used in these experiments. (Supported by the Natl. Science Fndn.)

1873. INHIBITION OF ANAMNESTIC RESPONSIVENESS BY X RADIATION. Andrew Thomson and David W. Talmage (intr. by C. W. Hammond). Dept. of Medicine, Univ. of Chicago, Chicago, Ill.

The primary response of rabbits to the I.v. injection of bovine serum albumin (BSA) in solution has been found to be a mixture of antibodies with widely different avidity for the antigen. Of 9 control animals given a 2nd injection of BSA 43 days after the 1st injection, 6 gave antibody responses significantly greater and of higher avidity than that found in primary responses. The amount of antibody and its average avidity were determined by the Farr ammonium sulfate test. The responses to the 2nd injection of antigen were found to correlate in increasing degree with 1) the antigen disappearance rate of the initial injection; 2) the height of the primary response; and 3) the antibody levels existing in the circulation just prior to the secondary antigen challenge. One group of rabbits was given 350 r total body X-radiation 13 days after the primary injection and challenged 30 days later. Three out of 5 animals did not have detectable antibody 8-15 days after the 2nd injection. The other 2 gave weak responses with an avidity of the primary type. A 2nd group of animals was X-radiated 41 days after the primary immunization and was given a secondary challenge 2 days later. Four of 7 animals gave detectable responses and in 2 the antibody level and the avidity were higher than that found in primary responses in unirradiated animals. These results indicate that X-radiation after completion of the primary response inhibits the anamnestic response more than X-radiation 2 days before the 2nd injection. It is possible that the earlier X-radiation prevents the development of anamnestic responsiveness, whereas the later X-radiation was given after this responsiveness had already been established.

1874. CHICK POTENCY TESTING OF POLIOMYELITIS VACCINES. RESPONSE OF CHICKS TO POLIOMYELITIS VIRUS PREPARATIONS. E. A. Timm,* E. Rope,* and I. W. McLean, Jr. Research Labs. Parke, Davis and Co., Detroit, Mich.

Antibody response in 1-day to 2-wk-old chicks against live and various killed preparations of poliomyelitis virus has been determined for varying doses, schedules and routes of injection. Antigens were inoculated undiluted and in half or full log (10-fold) dilution steps through 10^{-3}, 5 to 10 chicks/dilution. Chicks were bled from 3 5o 10 days following the final inoculation. In addition to mean serum titers determined for chicks receiving undiluted inocula, antigen dilution titers were calculated according to the method of Reed and Muench (Am. J. Hyg. 27:493, 1938) from the number of chicks responding at each inoculum dilution with demonstrable antibody at a serum dilution of 1:4. All chicks responded with demonstrable antibody to high titer when inoculated with undiluted preparations of all 3 types of polio virus, regardless of the age of the chick or the dose, route or schedule of inoculation. Day-old chicks were somewhat less consistent in response than 1-2k-old and older chicks. Providing the inoculum volumes were at least 0.5 cc, variation in route and schedule had very little effect on consistency of response, but a somewhat greater effect on the height of response to a given dilution. Antigen dilution titers appeared to be the best and most uniform measure of antigenic potency. The chick antigen dilution test proved to be a much more consistent test for antigenic potential than the current monkey potency test, and it was felt that it provided a measure of antigenicity more independent of the variation in the individual animal's ability to respond. In addition to the obvious cost advantage, the chick test has the additional advantage that chick sera is completely compatible with tissue culture cells.

1875. ANTIBODY DECAY RATES IN COLD-EXPOSED RABBITS. Ignatius L. Trapani (intr. by Justine S. Garvey). Dept. of Chemistry, California Inst. of Technology, Pasadena.

Rabbits weighing between 2 and 3 kg were maintained in a cold box at a temperature of -15°C. During a 6-wk adaption period the animals were periodically weighed and their serum protein concentration measured. Animals kept in the cold showed a 38.7% increase in weight and a slight increase in serum protein concentration as compared to a 22.2% increase in weight and a relatively unchanged serum protein concentration for the animals kept at room temperature. Four weeks later the animals were injected i.v. with 25 ml of a 2.75% homologous anti-BSA γ-globulin solution which was prepared from high titer serum by 1/3 saturation with ammonium sulphate at pH 7.8. The injected dose amounted to 686 mg of protein, of which 197 mg was antibody protein. The response of the cold-adapted animals was compared to animals which had been placed in the cold only 18 hr prior to the passive immunization and also to animals maintained at room temperature. A plot of the log percent antibody retained vs. time shows that approximately 46% of the injected antibody remains in the circulation after 24 hr and 25% at 72 hr for the cold-adapted animals. Thereafter the antibody decay rate appears to be relatively constant. If we extrapolate the straight part of the curve back to zero time and calculate the decay rate from that point, a value between 3 and 4 days is obtained as the half-life of the passively administered anti-BSA in cold-adapted or short-term exposed rabbits. These values indicate that cold-exposed rabbits exhibit a more rapid antibody decay rate than normal rabbits kept at room temperature. The mechanism of protein turnover rates in cold-adapted animals will be discussed. (Aided by Contract NR111-392 between the Office of Naval Research and the California Inst. of Technology.)

1876. GROWTH OF POLIOVIRUS IN TISSUE CULTURES OF RHESUS MONKEY TESTICULAR CELLS. Alfred A. Tytell and W. P. Fisher.* Dept. of Cytochemistry, Merck Sharp and Dohme Research Labs. West Point, Pa.

Rhesus monkey testicles were trypsinized by the continuous method of Rappaport. Twelve to sixteen organs yielded approximately 1 billion cells in 2 hr. These cells grew out well, yielding confluent sheets in 5-6 days in a lactalysate medium fortified with human or calf serum. The growth of cells in media fortified with human serum was predominantly fibroblastic while the growth in calf serum medium was predominantly epithelial. Strains of all 3 types of poliovirus (Mahoney, MEF-1, Saukett) grew on these cells rapidly reaching a titer of approximately $10^{-7.0}$. Poliovirus grew well in various media including Morgan and Parker's medium 199, lactalysate, Eagle's and even in Hanks' salt solution, although less rapidly. All 3 types of poliovirus gave very characteristic cytopathic changes. In general, maximal titers were achieved in approximately 48 hr. pH was very definitely a factor in the rate of growth of virus. Glutamine had no effect on the rate of virus growth or on the total infectious titer achieved. Cysteine did affect the rate of virus growth favorably. Plaque formation in sheets of rhesus testicular cells was demonstrated and well formed countable plaques were obtained in 72 hr. Although type I (Mahoney) poliovirus gave larger plaques, there was no characteristic morphology for each of the types. The rhesus monkey testicular cells were found to be especially useful in the assay of poliovirus.

1877. ENZYMATIC VARIANTS OF INFLUENZA VIRUS. Duard L. Walker and Billie L. Padgett (intr. by C. V. Seastone). Univ. of Wisconsin Med. School, Madison.

Mutants were isolated from cultures of Lee strain influenza B virus which were markedly less active than parent virus in enzymatic alteration of erythrocyte receptors and of soluble mucoproteins. These mutants were found to be stable in their characteristics on repeated subculture in embryonated eggs. They were indistinguishable from parent virus in antigenic make-up, pathogenicity for mice, in their capacity to agglutinate erythrocytes of several species and in several other characteristics not pertaining to actual enzymatic activity. Their enzymatic activity was very sensitive to calcium ion concentration, being enhanced by calcium and inhibited by removal of calcium while parent virus was not measurably affected by changes in calcium concentration. Although capacity to adsorb to and to agglutinate erythrocytes was destroyed at the same temperature for both parent and variant, the enzymatic activity of the variant was destroyed at about 47°C while that of parent virus was lost only on exposure to 55°C or above. Enzymatic activity of the variant was maximal at about 25°C and was inhibited by higher temperatures, although inhibition was apparently not due to thermal inactivation. Parent virus reached peak enzymatic activity at 37-40°C. Multiplication of variant virus in the allantoic membrane of chicken embryos was slightly slower than parent virus at 35°C and was markedly inhibited at 39°C although the parent virus multiplied well at 39°C. Inhibition of the variant at 39°C appeared due to a prolongation of the multiplication cycle.

1878. TYPE-SPECIFIC TOXINS IN GROUP A STREPTOCOCCAL LESION EXTRACTS. Dennis W. Watson Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.

Group A streptococci grown in the in vivo environment of rabbit skin produce a soluble factor not obtained in vitro. When tested in American Dutch rabbits, the factor is capable of potentiating the lethal and cardiotoxic properties of endotoxins and streptolysin O. In some respects, lesions observed in the tissues of these animals resemble those characteristics of the generalized Shwartzman reaction (Schwab, Watson and Cromartie. Proc. Soc. Exper. Biol. & Med. 82:754, 1953). Failure to develop a more direct assay for the factor has made additional characterization difficult. The present report describes 2 methods which measure the direct toxicity of the factor. In rabbits, toxin injected i.v. gives rise to a significant increase in serum transaminase as determined by Karmen et al. (J. Clin. Invest. 34:126, 1955). A 2nd useful response is the pyrogenic activity, as measured by rectal temperatures, produced concomitantly with the transaminase increase. Both responses become maximal in approximately 3 hr. Toxicity, as manifested by these criteria, is destroyed by heating at 60°C for 30 min at pH 7.2. Type-specific immunity can be demonstrated within 6-7 days by repeated i.v. injection of the toxin; this immunity has been obtained with extracts made with types 18, 28, 12 and 4. Even though no neutralizing or classical precipitating type antibodies have been demonstrated for these toxins, the high degree of specificity indicates that the tolerance observed is not of the Beeson type (Proc. Soc. Exper. Biol. & Med. 64:146, 1947). These methods of measuring direct toxicity should assist in establishing the nature and role of these toxins in the pathogenesis of group A streptococcal infections.

1879. IN VIVO ANTIGEN-ANTIBODY COMPLEXES. William O. Weigle (intr. by Frank J. Dixon). Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The sera of rabbits were tested for the presence of I^{131} antigen-antibody complexes at various intervals following both primary and secondary injections of I^{131} bovine serum albumin (I^{131} BSA). This was done by precipitating the globulins of the sera with 50% saturated ammonium sulfate. It was assumed that the I^{131} BSA present in circulating antigen-antibody complexes would precipitate with the globulin while the I^{131} BSA not so combined would remain in solution. In the primary response globulin bound I^{131} BSA did not appear in the sera of most of the animals until a rapid immune elimination of the antigen was evident. With an occasional rabbit in which there was an exceptionally slow immune elimination of antigen the globulin-bound I^{131} BSA was observed 1-2 days before an immune elimination was observed. At times 90% or more of the circulating I^{131} BSA was precipitated with 50% saturated ammonium sulfate (at 0°C). The sera of the same animals were also tested at various intervals for the presence of alkali precipitable material (Sternberger). Sometimes between 8 and 16 days following a primary injection of I^{131} BSA the sera of 5 out of 15 rabbits contained alkali precipitable material. Four of these animals contained both alkali precipitable material and ammonium sulfate precipitable I^{131} BSA-anti BSA complexes at the same time. However, in none of these sera which contained I^{131} BSA-anti BSA complexes was there a significant amount of I^{131} BSA found in the precipitates which resulted from alkali treatment. The sera of 7 animals receiving 400 r whole body radiation 2 days prior to injection of I^{131} BSA contained neither alkali precipitable material nor ammonium sulfate precipitable I^{131} BSA throughout the period of observation. (Supported by Atomic Energy Comm. Contract No. AT (30-1)-1205.)

1880. EFFECTS OF PERCHLORIC ACID UPON ELECTROPHORETIC AND CHEMICAL COMPOSITION OF HUMAN OROSOMUCOID. Henry E. Weimer and Elmer H. Rice (intr. by Charles F. Pait). Dept. of Infectious Diseases, Univ. of California Med. Ctr., Los Angeles.

Mucoprotein fractions isolated from human plasma by a procedure employing perchloric acid and ammonium sulfate have been shown to consist of 2 major and 1 minor electrophoretic components, designated MP-1, MP-2 and MP-3, respectively (Mehl et al. Proc. Soc. Exper. Biol. & Med. 72:106, 1949). A preparation, orosomucoid, obtained by a series of ammonium sulfate precipitations consisted of only 1 major electrophoretic component, MP-1 (Weimer et al. J. Biol. Chem. 185:561, 1950). The present study was undertaken to determine the effects of perchloric acid upon the electrophoretic and chemical composition of orosomucoid. Orosomucoid was incubated in 0.6 N perchloric acid at 25°C for 8 hr, followed by dialysis and lyophilization. Electrophoretic analyses were carried out at pH values of 8.6 and 4.5 in veronal and acetate buffers, respectively. Nitrogen, hexose, fucose, sialic acid and protein were determined. The presence of a 2nd electrophoretic component comprising 33% of the total area was apparent at pH 4.5. Inhomogeneity was noted at pH 8.6. The results of the electrophoretic and chemical analysis were compared with those of an untreated control preparation and with a mucoprotein fraction isolated by the use of perchloric acid and ammonium sulfate. Certain quantitative differences were found in the chemical compositions of the 3 preparations. The data suggest that a component similar to MP-2 may be derived from orosomucoid by exposure of the latter to perchloric acid.

1881. KINETICS OF IMMUNE HEMOLYSIS EMPLOYING Cr^{51}-LABELED SHEEP RED BLOOD CELLS. Roy S. Weinrach* and David W. Talmage. Depts. of Microbiology and Medicine, Univ. of Chicago, Chicago, Ill.

When sheep red blood cells which have been labeled with Cr^{51} are lysed, the radioactivity is released to the supernatant. The Cr^{51} system provides a sensitive and accurate measurement of immune hemolysis and permits the use of a greater excess of complement than is possible with spectrophotometric methods. When an excess of complement was added to red cells prior to the addition of hemolytic antibody, hemolysis proceeded at a constant rate so that a plot of log cells remaining vs. time was linear. When a series of such straight-line plots was obtained from serial dilutions of the same antiserum, the rate of hemolysis could be studied as a function of antibody concentration. If the logarithm of the hemolysin concentration is plotted against the log of the cells remaining after a fixed period of hemolysis (e.g. 30 min), the reciprocal of the slope of the resulting line is the apparent order of the lytic reaction. In most sera produced by 1 or 2 courses of immunization, the velocity of hemolysis was found to be proportional to the square of the hemolysin concentration. Natural hemolysins yielded orders between 1 and 2. With the γ_2 fraction obtained from a starch electrophoretic separation of a hyperimmune serum, the velocity of hemolysis was proportional to the 4th power of the hemolysin concentration. With the γ_1 fraction of the same serum, hemolysis varied according to the square of antibody concentration. These results indicate that with different types of antibody, 1, 2 or 4 molecules may participate in the hemolytic event.

1882. QUANTITATIVE STUDY OF DEVELOPMENT OF ANTIBODY RESPONSE OF CHICKENS TO BOVINE SERUM ALBUMIN. H. R. Wolfe, A. Mueller,* J. Neess* and C. Tempelis.* Dept. of Zoology, Univ. of Wisconsin, Madison.

Several hundred chickens were injected with a single dose of (40 mg/kg body wt.) bovine serum albumin at different ages. The circulating antibody nitrogen content was measured on the 8th day after the inoculation. The antibody production increased significantly until 22 wk of age. One of the breeds used was Arbor Acre White Rocks and though birds of 13 months of age gave higher mean titers than the 22-25-wk old animals, the differences were not significant because of the large variation in individual titers. The titers (μg antibody N per ml serum) for 1 group of Arbor Acres were 87, 205 and 360 μg nitrogen for 6, 12 and 22-2k old birds, respectively. One group of female White Leghorns ranged in age from 3 to 8 years and their response was higher than 1-1/2- and 2-1/2-yr birds but no statistical analysis was attempted because of the line differences. Only 6 birds failed to produce detectable precipitins; 5 of these were younger than 10 wk and 1 was 13 wk of age. (Supported in part by the Natl. Science Fndn.)

1883. ANTICOAGULANT ACTIVITY OF FUCOIDIN. Harold A. Wurzel* and Georg F. Springer. William Pepper Lab., Univ. of Pennsylvania, Philadelphia.

Purified fucoidin prepared from Fucus vesiculosus was found to possess anticoagulant activities (Springer et al. in press). Employing human blood in vitro, vacuum dried fucoidin possesses about 100% of the potency of heparin in the recalcification test and about 10% heparin activity in the whole blood coagulation inhibiting test. Fucoidin delays the action of thrombin on fibrinogen more than heparin. It is a less potent inhibitor in the 1-stage prothrombin test. Fifty milligrams

of fucoidin, given i.v. to adult rabbits, prolonged the clotting time for an average of 6 hr. From an average of 4 min, the recalcification time was usually prolonged to more than 30 min. In 5 to 12 animals, the clot retraction was impaired. In 1 rabbit a transient thrombocytopenia followed each infection of a standard dose. The prothrombin time uniformly was significantly delayed, but no change in the labile factor content was noted. The anticoagulant effects of fucoidin were unchanged on repeated injections. Toxic symptoms were not observed. Further in vivo studies assaying the antithrombotic and anticoagulant activities of fucoidin administered by various routes will be reported. (Aided by a grant from Ciba Pharmaceutical Prod., Inc., Summit, N. J.)

1814. **MULTIPLICITY OF COMPONENTS OF RAT KIDNEY ANTIGEN RESPONSIBLE FOR LOCALIZATION OF ANTI-RAT KIDNEY ANTIBODIES.** Yasuo Yagi* and David Pressman. Dept. of Biochemistry Research, Roswell Park Mem. Inst., Buffalo, N. Y.

It was shown previously that the soluble material obtained by the trypsin digestion of the saline insoluble sediment of rat kidney homogenate neutralizes localizing anti-rat kidney antibody. Evidence has not been found that the digest contains more than 1 type of neutralizing component and that there is more than 1 type of kidney localizing antibody. For example, heating of the digest at 100°C at pH 8 and 10 for 15 min destroyed its neutralizing activity almost completely, but only a part of it was destroyed at pH 6. Comparison of the neutralization curves obtained with original digest and with heated digest revealed a clear difference in slope, indicating the existence of at least 2 antigen-antibody systems. The antigen related to 1 is heat-resistant and to the other, heat labile. Further evidence for heterogeneity was found with the saline insoluble material obtained by treating kidney sediment with 60% trichloroacetic acid, centrifuging and diluting the supernate with water. The trypsin digest of this material neutralized localizing antibody but gave an antibody neutralization curve of a different shape than did the digest of the original sediment. Experiments with fractionated antibody also indicate heterogeneity of kidney localizing antibody. (Supported by Grant No. H-2092 of the Natl. Heart Inst., PHS.)

ABSTRACTS RECEIVED LATE

*An asterisk * following an author's name indicates "by invitation"*

AMERICAN PHYSIOLOGICAL SOCIETY

1885. METABOLIC RATES AND RECTAL TEMPERATURES OF ACTIVE AND "HIBERNATING" BLACK BEARS. Raymond J. Hock. Arctic Aeromedical Lab., Ladd AFB, Fairbanks, Alaska.

Perhaps the most widely reputed hibernator is the black bear. Field zoologists have long doubted that it did hibernate. To settle this doubt, and to fit the bear into the physiological scheme of hibernation, studies were made of several phenomena. The winter den temperature followed the outside air temperature, and varied from 1°-10°C above it. Rectal temperatures of a captive bear in its winter den taken at several times during the winter were found to be 34.0°C. Once the thermocouple stayed in place several days, the rectal temperature dropped slowly to 31.2°C. There was then no recorded temperature for an active black bear. Analysis of those since collected shows an average of 38.0°C for 10 active black bears, captive or wild, at all times of year. Thus there is only a small drop in rectal temperature in denning black bears, but there may be a significant metabolic saving due to this small decrease. Metabolic rate of an active black bear yearling in late fall was 0.36 ml O_2/gm/hr at 20°C, or thermoneutrality. In late winter, thermoneutrality ranged from 20° to -20°C, with lowest values at -10°C. Metabolic rate at this point was 0.28 ml O_2/gm/hr, while over the wider range it was 0.34. RQ for the whole series averaged 0.73. Using an air-tight den as a metabolic chamber, determinations of the denning metabolism of "hibernating" black bears are currently underway to determine whether the 7°C drop in rectal temperature will result in a 25% reduction in metabolic expenditure, as has been found in hypothermic humans.

1886. AVERAGE RESISTIVITY OF THE HUMAN BODY IN THE VICINITY OF THE HEART. Otto H. Schmitt. Univ. of Minnesota, Minneapolis.

Homogeneous torso model experiments have shown that the transfer impedance for the simple SVEC Y lead (head to leg) is remarkably constant throughout the entire region about the heart. Except for perturbations introduced by tissue inhomogeneities, this constancy should imply, by virtue of the electrical reciprocity theorem, that current introduced in this Y lead should be vertically directed and uniform in density throughout the chest. Equipotentials on the body surface thus delimit slices of torso of easily calculable impedance and it is possible to arrive at an average bulk resistivity if cross sectional area and mean separation of specific equipotentials is known. Using about 3 milliamperes of current at 500 cps and taking 5 ohm slices at about the heart level, mean resistivities were calculated for over 200 normal men and the distributions and average of these values determined. In many cases resistivity in the same individual at two different levels was determined. While a mean value of 530 ohm-cm with standard deviation of about 70 ohm-cm can be stated as typical, these values must not be taken out of context as they are subject to modest corrections for field form and heterogeneity. These corrections will be discussed. (Supported in part by grants from the Natl. Insts. of Health and the Minnesota Heart Assoc.)

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

1887. INTRACELLULAR BACTERIAL PEPTIDES. G. E. Connell and R. W. Watson (intr. by L. I. Pugsley). Div. of Applied Biology, Natl. Research Labs., Ottawa, Canada.

Numerous metabolically active intracellular peptides have been obtained from log-phase cultures of Pseudomonas hydrophila grown on glucose as the sole carbon source. Alcoholic extracts of washed cells were concentrated, extracted with ether and dialyzed. After fractionation of the permeates by high-voltage paper electrophoresis 5-7 ninhydrin-positive acidic, 1 neutral and 1-7 basic bands appeared. The peptides and amino acids from 3 of the acidic bands have so far been resolved into a total of 11 peptides containing from 5-11 constituent amino acids. Dialyzates from ether-extracted alcoholic extracts of samples taken at intervals up to 2 hr after the addition of uniformly-labeled C^{14}-glucose to a log-phase culture were radio-autographed after fractionation by high-voltage paper electrophoresis. All ninhydrin-positive bands were radioactive in less than 1 min, and no new bands appeared in the 2-hr period. Elution of acidic, neutral and basic fractions as 3 separate groups was followed by estimation of the weight and radioactivity of carboxyl-C before and after acid hydrolysis. Residual cell-protein had the highest total radioactivity even in the 1-min sample, with lower activity in the combined amino-acid plus peptide fraction. Specific radioactivities plotted against time gave parallel hyperbolic curves, with peptides below the curve for amino acids, and with cell protein still lower and almost linear. This evidence suggests rapid synthesis of peptides from amino acids.

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

1888. IMMUNOLOGIC RESPONSE OF MAN TO PURIFIED BIVALENT AB BOTULINUM TOXOID. Leonard F. Devine,* James T. Duff,* Mary A. Fiock* and George G. Wright. Chem. Corps, Fort Detrick, Frederick, Md.

Purified Cl. botulinum toxins were detoxified in the presence of formalin and adsorbed on aluminum phosphate. The product contained 3 Lf each of type A and type B toxoid and 7 mg of aluminum phosphate per ml. Four different schedules of immunization were investigated. A half ml of toxoid was given subcutaneously to 4 groups of volunteers at 0 and 8 wk, 0 and 10 wk, 0, 2 and 10 wk, and 0, 2, 4 and 6 wk. The groups consisted of 25 or more persons. A 0.5 ml booster of toxoid was given to each individual 1 yr after the initial injection, except that in the 0-10 wk schedule boosters were given after 6 months. Serum antitoxin titers of individuals were determined, after initial immunization and again at 8 wk after boosters, by neutralization

titrations in mice. On the basis of protection experiments in animals, titers of 0.02 units of type A and 0.005 units of type B were considered satisfactory. Prior to booster injections the 0 - 2 - 10 wk schedule produced the highest percentage of satisfactory titers. Antitoxin appeared sooner and was maintained longer than with other schedules. Satisfactory titers were found in the sera of essentially all persons after booster immunization. Prior immunization with type A toxoid markedly reduced the immunologic response to the type B antigen in the combined AB toxoid. Available data on response to univalent type A toxoid indicated that satisfactory levels of antitoxin were present 1 - 3 yr after boosters.

Notes

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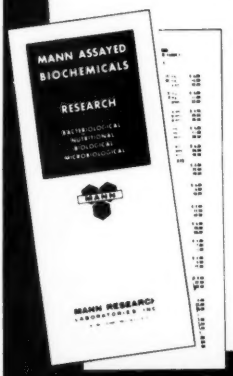

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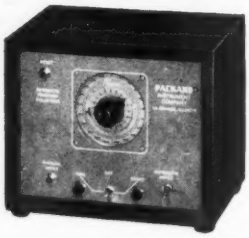

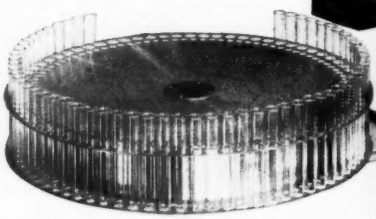

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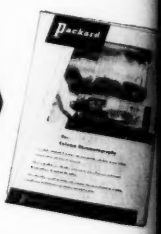

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